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ANALYTICAL METHODS IN BASE EXCHANGE INVESTIGATIONS ON SOILS

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It is now the consensus of opinion that in base exchange methods the soil chemist has an effective means for the investigation of many important soil problems. Procedures used by different investigators in this field have varied widely, even though the principles were supposed to be the same throughout. The writers do not intend to enumerate all the methods that have been used in base exchange work, or to enter into any discussion of the merits of each. The procedure that they have used in obtaining data published in another paper (13) will be described and salient points discussed, both as a matter of record which may be of interest to those who study the data presented and in the hope that an account of their experience may be of some help to others who may desire to work along similar lines.

AMMONIUM ACETATE AS A BASE EXCHANGE SALT

A neutral, 2 *M* solution of ammonium acetate was used by Prianishnikov (10) for the estimation of exchangeable potassium in soil; this investigator considered it the most satisfactory salt for the purpose, more especially because it is very easily decomposed by evaporation upon the steam bath, leaving only a slight residue of acetamide which may be volatilized at a slightly higher temperature or destroyed by a digestion with aqua regia. He recommended further study of its use for that purpose, but no one appears to have published anything further on the subject until one of us pointed out the advantageous features of a neutral solution of ammonium acetate for the estimation of all exchangeable cations in soil (11). The salt is almost ideal for the purpose, having numerous theoretical and practical points in its favor, summarized briefly as follows:

1. Ammonium acetate is a neutral salt, and its solutions have pronounced buffer properties around pH 7, as may be seen from the fact that the dissociation constants of both acid and base are practically equal and very small—about 1.8×10^{-5} at 25°C. In theory this is a very important point, because it means that hydrogen displaced from the unsaturated absorption complex of acid soils by the exchange reaction with the salt will be but slightly ionized, hence comparatively inactive. Because the H-ion concentration of the solution is not increased to any considerable extent by the accumulated product of the exchange reaction, replacement of NH_4 from the solution for H in the absorptive complex of the soil can proceed to a maximum.

A single treatment, therefore, should suffice not only for the extraction of all exchangeable cations but for the determination of total absorptive capacity as well. A preliminary treatment with an alkaline reagent has generally been considered essential for complete replacement of exchangeable hydrogen in preparation for the determination of total absorptive capacity. By the substitution of the neutral ammonium acetate for the acid-forming ammonium chloride as the agent for extraction of cations, the necessity for a pretreatment with an alkaline solution is avoided. It has long been known that there is danger of increasing the absorptive capacity of soil by a building up of base exchange complex by the action of strong alkali. Extraction of exchangeable bases by a solution not only neutral at the beginning, but with buffer properties adequate for the maintenance of neutrality has the further important advantage that solution of soil constituents not properly considered exchangeable, but which are dissolved in large amounts by the acid liberated by exchange in solutions of salts of strong acids, is largely or altogether avoided. The determination of "exchangeable" aluminum is an example. With respect to this element, it may be said that aluminum acetate is apparently soluble in a cold solution of ammonium acetate at pH 7, but $Al(OH)_3$ precipitates when warmed, and does not redissolve again. The acetate solution has no tendency to peptize freshly precipitated $Al(OH)_3$, as certain other organic ammonium salts have, hence any Al found in an ammonium acetate extract of soil may be presumed to have entered the solution by true exchange.

2. As has been mentioned, ammonium acetate is easily expelled by a simple evaporation during the analytical procedure. The decomposition does not require the use of any additional reagent and is not attended by active evolution of gas to cause loss in spray; separated salt does not creep up the walls of the beaker during evaporation; and the solution boils quietly without bumping.

3. Ammonium acetate is readily soluble in alcohol, permitting the removal by this solvent of the excess salt from the residue of soil, in preparation for the determination of total exchange capacity. Alcohol is superior to water for this purpose, as the tendency to hydrolysis of the ammonium absorption complex is less and alcohol also percolates through the soil more rapidly. It should also be practical to employ ammonium acetate in alcoholic solution as the extracting solution for calcareous soils, in order to minimize attack upon carbonates.

4. A neutral solution of ammonium acetate for base exchange work on soil is readily prepared from inexpensive reagents which may be obtained in high purity or easily purified to any required degree by redistillation.

LEACHING AS A MEANS OF EXTRACTING EXCHANGED CATIONS

Much of the recent work on exchangeable bases of soil has been done by digesting the soil with a portion of the extracting solution, filtering, and washing with more of the same solution. The final washing process is necessary for the removal of an important part of the total exchangeable cations which is in equilibrium with the extracted cations in the portion of solution used for the digestion. In effect, the process is the same as a leaching.

A point in favor of the preliminary treatment with ammonium acetate solution is that better advantage is thereby taken of the buffer properties of the salt. As will be understood from the discussion in a previous paragraph, in leaching an acid soil the exchange of hydrogen from the soil for the cation of the salt results in an accumulation of free acid in the solution. If this liberated acid is diluted by a considerable volume of ammonium acetate solution, the buffer properties of the latter will prevent any considerable increase in the H-ion concentration of the solution. On the other hand, in leaching an acid

soil the first portion of solution to pass through has acted upon successive portions of fresh soil, so that the hydrogen exchange will have been pushed to the limit and the reaction of the first few drops of solution may be as acid as that of the soil. No doubt this action is responsible for some attack upon minerals and solution of non-exchangeable bases, but it does not seem likely that it would ever lead to any great error with an acetate solution. All the work reported by the writers has been done by a leaching procedure, without preliminary digestion. The principal reason for this choice was that the simple leaching can be accomplished with minimum exposure to the atmosphere, which is best avoided on account of the possible effect upon the determination of hydrogen exchanged and ammonium absorbed by the soil. Absorption of CO_2 by the solution or loss of NH_3 from the soil was considered a factor of greater importance than a possible slight increase in amount of some cations extracted from the soil.

If many samples are to be examined, the simple leaching procedure requires less time and labor, as a number of units can be set up and the extraction allowed to proceed without attention. For only one or two determinations, a dispersion of soil in solution with a high speed stirrer (such as a malted milk mixer) followed by filtration and washing on a Buchner funnel, as described by Baver (2), is more rapid and has been found to give practically identical results so far as extraction of the principal exchangeable bases is concerned. Data obtained by T. C. Green in this laboratory from comparisons of the two procedures on a few samples have indicated, however, that the dispersion method gives considerably lower results than does the leaching method for NH_4 ion absorbed or for total absorptive capacity, so that some acid soils have been indicated to be over 100 per cent saturated with bases. Whether this was due to excessive exposure to the atmosphere during the procedure, possibly resulting in loss of ammonia from the fully saturated soil, was not determined.

There is a possibility that the long time of contact of soil with solution required by a gravity leaching procedure may result in some building up of exchange capacity even at pH 7, and so cause high results for exchanged hydrogen and total absorptive capacity. The possibility of variation in duration of contact with soils differing widely in degree of permeability is a disadvantage of leaching. As in the determination of other "available" soil constituents, the results obtained in base exchange studies depend to a considerable extent upon adherence to a given set of conditions. If the soil is very finely ground, leaching will be very slow, and higher values for most determinations will be obtained. It is a question how much of the increased action noted with ground soils is due to more efficient contact of solution with particles formerly protected in aggregates, how much is due to increase in time of action, and how much is due to exposure of fresh surfaces of undecomposed minerals as the result of the grinding.

Experiments in which 100-gm. portions of acid Wooster silt loam surface soil free from carbonates, air dry, and sieved through 2-mm. mesh, were leached

with 250, 500, 1,000, and 2,000 ml. portions of neutral *N* ammonium acetate have indicated that by the procedure about to be described practically maximum indications for bases exchanged and exchange capacity are obtained by leaching with 500 ml. of the solution. The quantity regularly used has been 750 ml., requiring 18 to 24 hours to percolate through the above described soil. Experience with other soils has indicated that this volume is usually sufficient, with the possible exception of some very heavy clay soils. The quantity of sample used (100 gm.) is larger than that taken by many other investigators in this field. Large samples were considered desirable on account of the low exchange capacity of our soils, and the very small amounts of the alkalies present. A leaching procedure is especially well adapted to work on large samples. Data from analyses of extracts obtained by leaching duplicate 100-gm. samples of 2 mm. acid Wooster silt loam with 750 ml. neutral *N* ammonium acetate and chloride, respectively, are compared in table 1. The most significant differences are in Al dissolved from the soil and in NH_4 absorbed from the solution. Exchangeable H was not determined, but from the data

TABLE 1

Comparison of exchangeable bases in Wooster silt loam as extracted by leaching with neutral ammonium acetate and chloride solutions

Milligram-equivalents per 100 gm. air-dry soil

	Al	Mn	Ca	Mg	K	Na	NH_4 absorbed
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
Acetate	0.16	0.12	1.16	0.32	0.12	0.44	6.76
Chloride	2.56	0.28	1.04	0.40	0.12	0.40	3.96

presented a good idea of what it should have amounted to may be formed. The differences between NH_4 absorbed and the sum of bases other than Al extracted in each case indicate that the chloride leaching displaced about 1.8 m.e. H, but the acetate leaching more than twice as much, 4.6 m.e. There is also a considerable difference in the amounts of Mn extracted by the two reagents.

METHODS EMPLOYED

Leaching Solutions

Ammonium acetate sold as a reagent is an acid salt. The reaction of a *N* solution is usually near pH 5, indicating that there is about one mol of free acetic acid to two of the ammonium salt. It is less trouble to prepare a neutral solution from equivalent solutions of acetic acid and ammonia than to attempt to neutralize a solution of the crystals. Three 5-gallon bottles should be at hand. In the first, dilute 2,500 ml. aqua ammonia, sp. gr. 0.9, to 18 liters. Transfer 25 ml. of the solution with a pipette and dilute to 250 ml. in a volume-

tric flask. Transfer an aliquot of this to excess standard HCl and titrate excess acid with 0.1 *N* NaOH and methyl red. Dilute the solution in the bottle to exactly 2 *N*. In a second bottle, dilute 2,100 ml. acetic acid, 99.5 per cent, to 18 liters. Titrate an aliquot of this solution diluted in the same way with the same NaOH and phenolphthalein, and make the solution exactly 2 *N*. In the third bottle, mix 9 liters of each solution. If the solutions were accurately prepared, the mixture will be at pH 7.0. If it is desired to verify this, transfer 100 ml. of the mixture to a 250 ml. beaker, bring to 25° C., add about 0.1 gm. quinhydrone and determine the reaction potentiometrically. If the reaction is not at the desired point, add from a burette the solution required, diluted 1:10, until the reaction is correct. From this titration, calculate the addition to the mixture to bring to the desired reaction. As the comparatively strong acetic acid and ammonia solutions when mixed contract about 25 ml. for each 2 liters, a further dilution to compensate for this will be required to make the solution normal. The writers have considered it desirable to have the leaching solution slightly alkaline in order to ensure maximum absorption of the ammonium ion, and have adjusted it to pH 7.07. Opinions may differ as to the desirability of this, but it is probably not of great importance.

For leaching excess ammonium acetate from the soil prior to the determination of absorbed NH_4, 80 per cent alcohol has been employed. This was prepared by mixing 16 volumes of 95 per cent ethyl alcohol (so-called "Cologne spirit") with 3 volumes of water. As alcohol of this grade is usually slightly acid, it has been our practice to add sufficient ammonia to cause the color with bromthymol blue to match that of the acetate solution. This neutralization with ammonia is considered desirable, as the removal of NH_4 from the saturated soil by the acid alcohol is thereby prevented and the tendency of the ammonium base absorbent complex to hydrolyze probably reduced. The actual amount of ammonia added is so small that it could scarcely have any noticeable effect in any other way.

Leaching apparatus

Special Pyrex filter tubes 4 cm. inside diameter, 17.5 cm. long including stem 5 cm. long have been employed as containers for the soil during percolation. A 40-mm. perforated porcelain plate rests on three indentations in the glass and is secured and made tight at the edges by means of ceresine wax or "Picein"[1] melted and caused to run around the joint by warming and turning the apparatus. A neatly cut circle of filter paper covers the holes in the plate. The leaching solution is delivered from and received in liter Erlenmeyers, connected with a siphon and an air return tube as shown in figure 1. A thin coating of the cement applied to the lower part of the taper on the no. 9 rubber stopper which closes the top of the percolator is advisable as insurance against

[1] A German preparation obtainable from Wilkens-Anderson Co., Chicago. It is very well adapted to this use, as it melts easily, adheres to glass and porcelain, and is tough, strong, and resistant to alcohol.

any leakage at this point. To fill the percolator, the paper circle is first wetted and spread on the porcelain plate. The dry soil is poured in in several portions, each being worked down somewhat with a spatula, to reduce shrinkage when the solution wets the soil. The apparatus is connected, except the air return tube and cap for escape of air at the top of the percolator. By blowing into the air return tube the siphon is started, and the apparatus then closed. When the ammonium acetate solution has percolated, the apparatus is disconnected, the percolate removed, and 500 ml. alcohol passed through the soil in the same way, to remove ammonium acetate from the soil.

FIG. 1

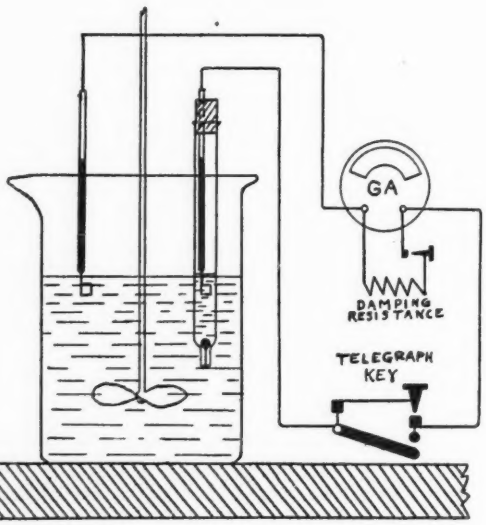

FIG. 2

FIG. 1. APPARATUS FOR LEACHING SOIL

FIG. 2. APPARATUS FOR POTENTIOMETRIC TITRATION

Exchanged hydrogen

The determination of exchanged hydrogen should be carried out as soon as possible after the solution has been removed from the receiving flask. An 800-ml. Pyrex beaker containing the percolate is placed under the apparatus shown in figure 2. The small vessel for the comparison electrode is made from a thin test tube with short tube fused to the bottom and a glass bead with tail ground in with carborundum powder; the ground joint serves as a salt bridge. The two electrodes should be cut from the same piece of platinum foil and fused in glass tubes, with mercury for connection to the wires leading to the galvanometer. The electrodes are prepared for use by heating in H_2SO_4-CrO_3,

washing, and ignition in alcohol flame. When used for a succession of determinations on the same day, they may be kept in acetate solution when not in use. Some of the original acetate solution is placed in the comparison vessel, a little quinhydrone added, and the stopper bearing the electrode inserted. This arrangement is supported in the solution so that equality of temperature is ensured. About 0.5 gm. quinhydrone is added to the solution in the beaker and the stirrer started. A quick tap of the key indicates by the deflection of the galvanometer (L. & N. Type R, with 500-ohm coil and provided with damping resistance) whether or not the solution is more acid than it was at the beginning. If acid, 0.2 *N* NH_4OH is added from a burette as rapidly as possible with frequent observations of the effect upon the indications of the galvanometer. As this becomes less, the ammonia is added more slowly, and finally the connection through the galvanometer is made permanent and the spot of light brought to the zero of the scale by additions of drops of ammonia. By standardizing this procedure and working as rapidly as possible, excellent results have been obtained, judged from agreement of duplicate determinations at different times. Titrations of 40-ml. portions of 0.2 *N* acetic acid in 750 ml. neutral acetate have indicated that although there is a plus error averaging 2.2 ml. 0.2 *N* NH_4OH, this is constant to within 0.5 ml. This average correction has been deducted in our work. Fresh solution and quinhydrone must be placed in the comparison electrode vessel for each determination. Following the titration, the beaker with the solution is set on the steam plate to evaporate.

Purification of the residue of salts

The residue from evaporation contains quinhydrone, as well as the acetates of the bases extracted from the soil. Most of the organic matter is volatile without difficulty and ignition in the beaker, similar to the procedure of Bray and Willhite (3), has been applied in the case of a few extracts from limed soils. According to our experience, the ignition in glass has not caused any noticeable contamination with acid-soluble matter, but many small pieces of glass have been detached by the action of the fused salts and the beaker is very likely to break under such severe treatment. With a little extra trouble it would be possible to conduct the final stages of the evaporation and the ignition in a platinum dish with greater safety. The organic matter has usually been destroyed by acid treatment in the beaker as follows:

Add to the residue from evaporation of the acetate extract 15 ml. aqua regia, cover, and evaporate. Add about 15 drops conc. H_2SO_4 and 5 ml. HNO_3 and boil off the acid, keeping the breaker grasped with a Chaddock clamp in constant motion to prevent the acid condensed on the watch glass from dropping on the heated bottom. As soon as the nitric acid has been expelled and heavy fumes of sulfuric acid appear, remove the watch glass and heat the upper part of the beaker as well as the bottom to volatilize organic matter and most of the excess sulfuric acid. Clean the watch glass in the same way and replace it. If the residue is still black, add a little more nitric acid and repeat. This will generally be sufficient. Take up with 15 ml. 5 *N* HCl and 150 ml. water and boil to get $CaSO_4$ into solution. Filter into a 250-ml. Erlenmeyer, wash, and discard the filter.

Ammonium sulfide precipitation

Neutralize the solution in the flask to methyl red with ammonia, add 5 ml. 5 *N* $(NH_4)_2S$, fill to the neck, and stopper. Let stand in a warm place over night. Filter through 9 cm. paper into a 400-ml. beaker and wash rapidly with a cold solution of 10 gm. NH_4Cl and 5 ml. $(NH_4)_2S$ in a liter of water, to prevent MnS being reoxidized and entering the filtrate. Roll the paper with the precipitate of MnS and $Al(OH)_3$ into a ball and preserve in the flask.

Calcium

On account of the wide difference in amount of Ca and Mg in leachings from limed and unlimed soils, different procedures have been used for the determinations. In the case of soils containing little of these elements extracted by ammonium acetate solution, the filtrate from the sulfide precipitate was set on the steam plate and allowed to evaporate to about 75 ml. There should be no precipitate, except a slight deposit of sulfur adherent to the glass, as the result of this evaporation. Acidify with a drop or two of HCl and add dropwise to the boiling solution 5 ml. saturated $(NH_4)_2C_2O_4$. If no precipitate appears, neutralize with ammonia. If the precipitate is heavy, it is probable that more oxalate will be necessary, and 5 ml. more may be added. Digest for about an hour on the steam plate, with occasional addition of a drop of ammonia to keep alkaline. Filter on a small gooch with a paper circle cut to fit, receiving filtrate and first hot water washings in a 150-ml. beaker. When washing is complete, place the crucible with the precipitate in the beaker, add 50 ml. water and 5 ml. conc. H_2SO_4, heat to 70°C., and titrate with 0.05 *N* $KMnO_4$.

Magnesium

Evaporate the filtrate from the oxalate precipitation to about 60 ml. and cool. Precipitate the Mg by addition of 5 ml. or more *N* $(NH_4)_3AsO_4$ followed by dropwise addition, with constant stirring, of conc. NH_4OH in volume equal to one-tenth that of the solution. Set the beaker in a large desiccator jar, or use some other effective means to prevent the escape of the ammonia, and let stand in a cool place until the next day. Filter as in the case of Ca, but wash with 1:10 cold NH_4OH, receiving the filtrate and first washings in a 150-ml. beaker. Drain the beaker thoroughly and draw air through the filter long enough to remove the last drop of wash solution. Place the crucible with the precipitate into the beaker and set aside for an hour to dry, but do not heat to hasten drying. When air dry, all free ammonia will have escaped. Add a small excess of 0.1 *N* HCl and a drop of methyl orange indicator and titrate excess acid, as in Handy's well-known method for Mg (6).

When both Ca and Mg are high, as was the case with our limed soils, the successive precipitation and titration of both without a separation, as originally proposed by Fox (5) but with some change in details in the Mg determination, has been found to be satisfactory. To the filtrate from the sulfide precipitate,

about 300 ml. in a 400 ml. beaker, add a slight excess of HCl and 0.5 gm. oxalic acid. Heat to boiling and precipitate the Ca by very gradual addition of dilute NH_4OH until the odor persists. Let stand 5 minutes, then test for complete precipitation by dropping in a little $(NH_4)_2C_2O_4$ solution. If the precipitation is not complete, it must be made so. When all the Ca is precipitated, add a moderate excess (5 to 15 ml.) of *N* $(NH_4)_3AsO_4$ and ammonia as previously described, after cooling. Filter and wash as if precipitate was of $MgNH_4AsO_4$ only, but titrate CaC_2O_4 by suspending the precipitate in 100 to 200 ml. water, adding about 5 per cent volume conc. H_2SO_4, heating to 70°C. and using 0.2 *N* $KMnO_4$ in this case. As soon as this titration is finished, add 5 gm. KI to reduce arsenate to arsenite and set over a burner. Add Na_2SO_3 very carefully[2] to discharge the iodine color. Continue the heating 15 minutes, adding more sulfite as may be required. Set in water to cool and neutralize most of the acid by careful addition of strong NaOH solution, but leave the solution acid. Add a little starch solution and 0.1 *N* iodine to color, then destroy this color with a trace of sulfite. Add excess dry $NaHCO_3$ and more starch solution and titrate to a definite blue color permanent for 5 minutes, with 0 1 *N* iodine solution, which is conveniently made by dissolving 25 gm. KI in 100 ml. cold water in a 500-ml. volumetric flask, and adding 10 ml. concentrated HCl in 100 ml. water. To this acidified iodide solution add from a pipette 250 ml. 0.2 *N* $KMnO_4$ with constant swirling to mix, and make to volume with water. The normality of this solution with respect to Mg will be exactly half that of the $KMnO_4$ with respect to Ca.

Alkalies

To the filtrate from Mg precipitation and first washings, in a 150- or 250-ml. beaker, is added 12 drops of conc. H_2SO and the solution evaporated as far as possible on the steam plate and still avoiding loss by spitting when the salts begin to separate. The evaporation may be completed and the salts finally dried in an oven at 110°C. At this stage of the work there is considerable danger of loss by creeping over the edge of the beaker, so that it will be well to watch it.

The beaker with the dried residue of salts is set in an electric crucible furnace in a good hood and heated below redness to expel ammonium salts and arsenic. During this ignition, the flame of a burner held in the hand should be directed at intervals upon the exposed rim of the beaker and the watch glass covering it, in order to drive off salts which would otherwise sublime there. When the ammonium salts have been expelled the residue should be nearly white, and aside from insoluble matter (mainly silica) consists of the sulfates of the alkalies. This residue is taken up with a little water and a drop of HCl and boiled for a few seconds to dissolve it. The solution is made alkaline with a drop or two

[2] Addition of sulfite in excess may cause a yellow color to appear, which is likely to be mistaken for that of iodine. That an excess of sulfite is present will be evident from the odor of the solution.

of ammonia and a little sulfide, and filtered through a small paper into a weighed 100-ml. Pyrex beaker. This solution is evaporated, ignited, and cooled for 15 minutes in the air before being weighed, as a precaution against error from electrification of the glass beaker.

For each centigram of sulfates, 1 ml. $PtCl_4$ solution (contains 10 gm. Pt a liter) is to be added and the solution evaporated just to dryness. After the residue is cool, excess $PtCl_4$ is dissolved from the crystals of K_2PtCl_6 with 80 per cent alcohol and the crystals and any insoluble matter in the beaker are transferred to a small Gooch with paper and asbestos, previously dried and weighed. After sufficient washing with the alcohol to remove excess Pt, sulfates are removed by about six washings with half saturated NH_4Cl and the latter removed by a final thorough washing with alcohol. All the filtrates are collected in the same beaker and set aside. The alcohol is allowed to evaporate from the Gooch by standing in the air for several hours, with a final drying at 110°C. before weighing. The potassium salt is removed from the Gooch by washing with hot water and the crucible again dried and weighed. The loss in weight noted between the second and third weighings represents K_2PtCl_6 and is calculated to K, whereas the gain in weight of the third over the first weight represents insoluble matter, which is to be deducted from the weight of alkali sulfates, as determined from the increase in weight of the beaker. The weight of K_2SO_4 calculated from the weight of K_2PtCl_6 found is further deducted, leaving the weight of Na_2SO_4.

All the errors of the analysis fall upon the sodium determination, hence it is well that the analyst satisfy himself that the previous separations were complete. Manganese and magnesium are most likely to escape precipitation at the proper point; the presence of the former in the alkali sulfates will be shown by a brown color, but the latter is not so easily detected. If these were present, they will remain in the solution from which excess Pt was precipitated by NH_4Cl. The Pt salt is filtered off and washed with a little more NH_4Cl solution to dissolve sulfates. The clear alcoholic filtrate is tested first with a little sulfide for Mn, then with oxalate for Ca, and finally with arsenate for Mg. The separations from this alcohol solution are rapid and the testing does not require much time. If appreciable quantities are found, determination as sulfate or calculation to that form and correction of the weight of alkali sulfates by the proper deduction are necessary.

Aluminum

The filters containing the main sulfide precipitate as well as any that may have been recovered while working with the alkali sulfates, are removed from the flask and ignited in a platinum crucible. The ash is fused with $KHSO_4$ at red heat and the melt dissolved. In the meantime 5 ml. conc. HNO_3 has been evaporated in the flask to remove the chloride and the flask then rinsed with a little HNO_3 and water. This solution and that of the bisulfate melt are united in a 200-ml. volumetric flask and made to volume. The solution is

filtered through a dry paper, and half of it taken for the determination of Al by an adaptation of Burgess' (4) method; to the 100-ml. aliquot in a 150-ml. beaker are added a drop of alizarin-S indicator and ammonia to a brownish red tint, indicating pH 5. Then are added successively 2 ml. *N* $(NH_4)_2HPO_4$, 1 ml. *N* 5 HCl to clear, and 2.5 ml. *N* $Na_2S_2O_3$; and this mixture is boiled slowly while covered 15 minutes. After the addition of 5 ml. 5 *N* $NH_4C_2H_3O_2$ at pH 5, the boiling is to be continued for 15 minutes longer, then filtered and washed with hot water. If much Mn is present, it will be necessary to dissolve the $AlPO_4$ from the precipitate with hot dilute HCl, catching the solution in the same beaker and repeating the procedure in order to get a good separation. The precipitate of $AlPO_4$ and S is finally ignited in a porcelain crucible and weighed as $AlPO_4$.

Manganese

The remaining 100 ml. of solution of bisulfate fusion, or an aliquot if Mn is high, is used for this determination. It is transferred to a 250-ml. beaker and 5 ml. conc. HNO_3 and 25 ml. $AgNO_3$ (1.2 per cent solution) are added, followed by 1 gm. $(NH_4)_2S_2O_8$. The beaker is set upon the steam plate to heat slowly to 90°C., at which point the maximum permanganate color should be developed. The solution is cooled quickly, Ag precipitated by the addition of 25 ml. NaCl (0.4 per cent solution), and without filtering titrated to disappearance of color with standard Na_2HAsO_3 solution as described by Scott (14).

Exchangeable Ammonium

A procedure similar to that described by Merkel (8) was employed for the estimation of this constituent; 100 gm. dry soil, as used for the ammonium acetate leaching, is weighed out and transferred to a 500-ml. Erlenmeyer The flask is filled with 0.1 *N* HCl and shaken at short intervals for 5 minutes, then the suspension filtered on a 10-cm. Buchner. The filtrate is transferred to a liter volumetric flask, and the soil washed with more of the acid until the total volume is 1 liter. An aliquot of 500 ml. is transferred to a copper flask, a slight excess of alkali added, and the ammonia distilled into excess standard H_2SO_4, and titrated with 0.1 *N* NaOH and methyl red indicator. In some instances, the determinations by this method were checked by the method of McLean and Robinson (9), employing a solution of NaCl for extracting NH_4 from the fresh soil and distilling off NH_3 as described in the foregoing. As good agreement was obtained, either method seems equally reliable.

Ammonium absorbed

The method described by Kelley and Brown (7), with slight changes, has been used for the determination of NH absorbed from ammonium acetate by the soil, or total absorptive capacity. Following the leaching with alcohol to remove acetate, as much of the alcohol as possible is drawn from the soil in the percolator by application of suction, but care being taken not to dry the

soil, which would result in the loss of considerable absorbed NH_3. The moist soil is turned from the percolator into a weighed beaker with cover, and after thorough mixing with a spatula the weight of damp soil is determined. One fourth of the soil is transferred to a copper flask, 400 ml. water with a few drops of capryl alcohol and mineral oil added to reduce foaming during distillation, and 50 ml. strong NaOH added, and the NH_3 distilled slowly for 45 minutes. A similar determination on 25 gm. of the original soil has been applied as a blank. It would have been better to have removed the small amount of exchangeable NH_4 from the blank sample by extraction with acid or salt solution although the error introduced by this neglect was insignificant and can be corrected by other data obtained The blank is quite large, because of decomposition by the drastic treatment of organic matter containing nitrogen. In our experience it has been quite constant for a particular soil, and this method is considered satisfactory for the determination if carried out with some precaution to ensure uniform distillation period for all samples.

Correction for carbonates dissolved

The residue of percolated soil after removal of the portion for determination of NH_4 absorbed may be dried and ground for determination of carbonate dissolved from a calcareous soil during the leaching process, comparing the data so obtained with that from examination of the original sample. The apparatus we have used for the determination of carbonates in soil by boiling with 1:50 HCl *in vacuo* has been described (1), also the absorption of CO_2 evolved in excess $Ba(OH)_2$ and titration (12).

Blanks

In consequence of the large volume of solution which is used for the extraction of the exchangeable bases from the soil, appreciable amounts of impurities are introduced, even when the best reagents obtainable are used. In our experience, a small amount of calcium and a considerable amount of sodium have invariably been found in blank determinations with the regular procedure, but without soil. It has been noticed that these blanks are larger when old aqua ammonia has been used for the preparation of the ammonium acetate solution for leaching the soil, or when the acetate solution had been kept for a considerable time in a bottle of common glass.

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STUDIES OF SOILS IN THE PLASTIC STATE

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The work of Atterberg and Johannson upon soil consistency published between 1911 and 1916 (1, 2, 10 and numerous other papers) has lately received attention in this country and in England. Atterberg's methods have been reviewed at some length by Russel and Wehr (15). These authors gave detailed descriptions of their adaptations of the Atterberg methods, and determined the Atterberg numbers for the various consistencies of a number of Nebraskan soils. Baver more recently has given the effects produced by controlled base replacement upon some of the Atterberg numbers (3). The "lower liquid limit" and "lower plastic limit" are commonly determined in the preliminary examination of subgrade material for road construction in the Bureau of Public Roads of the U. S. Department of Agriculture. Wintermeyer (20) has described the methods for obtaining these values. Their significance from the standpoint of the identification and evaluation of soils for construction material has been discussed by Terzaghi (18). As reported by Davis (6) some of the Atterberg numbers have been determined for certain soils collected by the U. S. Bureau of Chemistry and Soils. Haines, working at the Rothamsted station, examined soil cohesion and the pressure of fluidity for certain clay separates, ball clays, and a few soils (9) as part of an investigation into the mechanical properties concerned in cultivation. Methods very similar to those of Atterberg and Johannson were used for the cohesion measurements. A few years after the appearance of the first (1, 2) of Atterberg's papers on soil consistency, Kinnison of the U. S. Bureau of Standards compared the order of relative plasticity as determined by the Atterberg method, with that obtained by some of the then used methods for measuring plasticity in America, for a variety of shales, kaolins, and clays (11). He concluded that no single method is sufficient to classify clays as to their plasticity, and that each included some results out of harmony with the observed facts as recorded from common experience with the clays studied. He considered that the most reliable rating for the series was to be obtained from the product of the plasticity number, the water of plasticity, and the water vapor adsorbed over 10 per cent sulfuric acid.

DESCRIPTION OF SOILS USED

It was the purpose of the present investigation to examine the relation of the moisture content at the plastic consistency to some of the other properties of a fairly large number of Californian surface soils. Forty-three soils, members of 23 soil series, and 1 unnamed lacustral soil, were used. In addition 10 soils from India, members of 8 soil series, were included in the study. The Californian soils consist of primary, or residual, and secondary soils. The primary soils have developed from parent material which includes basic and meta-

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TABLE 1

Description of soils used

(After Shaw)

NUMBER	DIVISION	FAMILY	STAGE	GROUP	SERIES AND TYPE	AREA
			Californian soils			
			Mineral soils—primary			
			1. Without lime accumulations in the soil profile			
1	B	Claypan	Semimature	Dark	Climax clay adobe	Ukiah
2	B	Claypan	Semimature	Dark	Climax clay	Healdsburg
3	B	Claypan	Semimature	Brown	Olympic clay adobe	Bay Region
4*	B	Claypan	Semimature	Red	Aiken stony loam	Grass Valley
A(1)*	B	Claypan	Semimature	Red	Aiken clay loam	Placerville
			2. With lime accumulations in the soil profile			
5	C	Claypan	Semimature	Dark	Diablo clay loam adobe	Santa Maria
			Mineral soils—secondary			
			1. Without lime accumulations in the soil profile			
6*	A	Iron-silica hardpan	Mature	Red	San Joaquin sandy loam	Sacramento Valley
7	B	Unknown	Recent	Dark	Pit clay adobe	Big Valley
8	B	Unknown	Recent	Dark	Pit clay loam	Big Valley
9	B	Unknown	Recent	Brown	Vina clay adobe	Big Valley
10*	B	Unknown	Recent	Brown	Vina fine sandy loam	Chico
11*	B	Unknown	Recent	Brown	Vina fine sandy loam	Chico
12	B	Unknown	Immature	Brown	Gould clay adobe	Big Valley
13	B	Unknown	Recent	Brown	Buntingville clay loam	Big Valley
14	B	Unknown	Recent	Brown	Buntingville clay loam	Big Valley
15	C	Claypan	Recent	Brown	Wapato silty clay loam	Willits

16	C	Claypan	Recent	Dark	Dublin clay	Ukiah
17	C	Claypan	Recent	Dark	Dublin clay adobe	Bay Region
18	C	Claypan	Recent	Brown	Yolo silty clay loam	Ukiah
Yc(1)	C	Claypan	Recent	Brown	Yolo clay	Davis
Ycl(1)	C	Claypan	Recent	Brown	Yolo clay loam	Davis
19	E	Claypan	Recent	Brown	Bayside silt loam	Eureka
20	E	Claypan	Recent	Gray	Coquille silty clay loam	Eureka
		2. With lime accumulations in the soil profile				
21	B	Lime and lime-iron hardpan	Mature	Dark	Gazelle silty clay loam	Big Valley
22*	B	Lime and lime-iron hardpan	Young	Brown	Nord fine sandy loam	Chico
23	C	Lime hardpan	Semimature	Dark	Montezuma clay adobe	Santa Maria
24	C	Lime hardpan	Young	Brown	Willows clay	Cortena
25*	C	Lime hardpan	Young	Brown	Willows clay	Cortena
26	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
27*	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
28*	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
29	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
30*	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
31	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
32*	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
33	E	Lime hardpan	Immature	Gray	Lahontan silty clay loam	Honey Lake
34	E	Lime hardpan	Immature	Gray	Lahontan clay adobe	Honey Lake
35	E	Lime hardpan	Immature	Gray	Lahontan clay	Honey Lake
36	E	Lime hardpan	Recent	Brown	Holtville clay	Brawley
37	E	Lime hardpan	Recent	Brown	Holtville silty clay loam	Brawley
38	E	Lime hardpan	Recent	Brown	Imperial clay	El Centro
39	E	Lime hardpan	Recent	Brown	Imperial silty clay loam	Brawley
40					Lacustral clay	Bay Region

TABLE 1—*Concluded*

NUMBER	DIVISION	FAMILY	STAGE	GROUP	SERIES AND TYPE	AREA
				Indian soils		
				Mineral soils		
41					Vindhyan loam	Shivpuri
42					Shivpuri loam	Guna Rd.
43*					Gwalior silt loam	C. E. Farm, Gwalior
44					Shivpuri loam	Gujarda
45*					Vindhyan loam	Shivpuri
46					Malwa clay loam	Tajpur Ujjain
47					Malwa clay loam	C. Farm Ujjain
48					Sipra clay loam	Dhondarka
49					Singanwas clay loam	
50					Nahri clay loam	Fatchpur

* Not surface soils.

Explanation

1. *Division* refers to geological origin of parent material.
A—Soils composed of materials from disintegrated and decomposed igneous and metamorphosed igneous rocks high in quartz.
B—Soils composed of materials from disintegrated and decomposed igneous and metamorphosed igneous rocks low in quartz.
C—Soils composed of materials from disintegrated and decomposed sandstone and shale rock sources.
E—Soils composed of materials from disintegrated and decomposed mixed or undetermined rock sources.
2. *The Family* is based on the character of the profile shown by the fully weathered mature soil.
3. *The Stage* is based on the maturity of the soil.
4. *The Group* is based on the color of the surface soil.
5. The term *Series* has its usual significance.
6. The textural description is based on the surface soil.

morphosed igneous rocks, sandstones, and shales. The secondary soils have developed from unconsolidated sediments derived from a still wider variety of rocks.

They were collected at altitudes varying between 200 feet below sea-level in

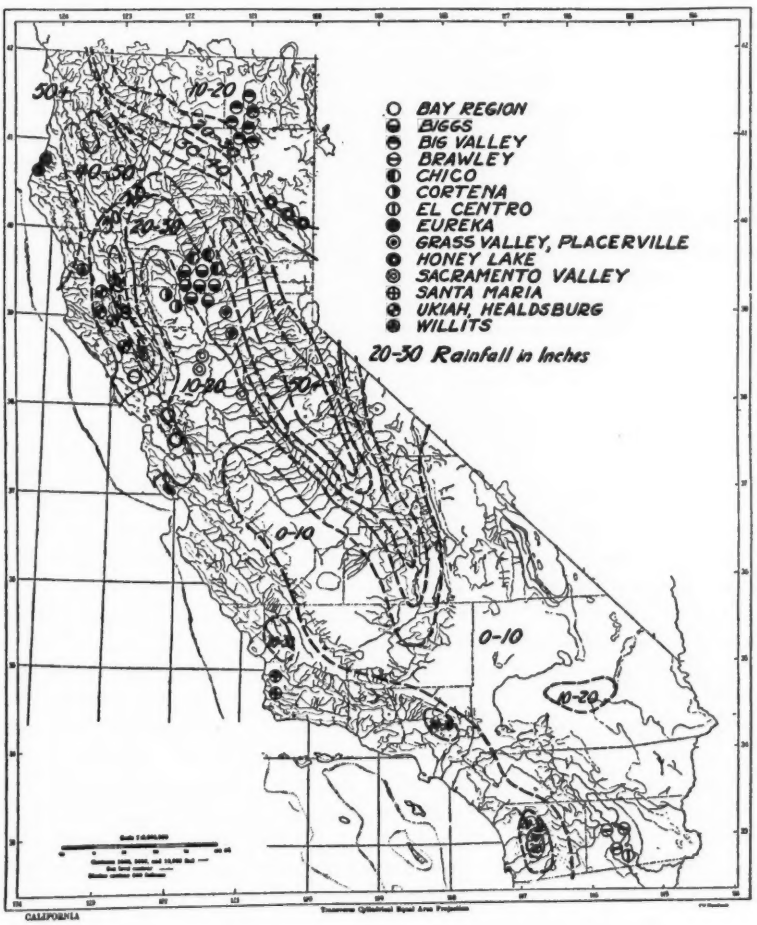

FIG. 1. OUTLINE MAP OF CALIFORNIA SHOWING LOCATION OF SOILS

Imperial Valley and 4,300 feet above sea-level in Big Valley. They represent climatic conditions with annual precipitations of from less than 4 to over 50 inches, and temperatures which are practically frost free to those having frosts during 10 months of the year. They were taken from the collection of soil

samples made by the University of California and the U. S. Dept. Agriculture Bureau of Chemistry and Soils in the course of the soil survey of California. This collection includes representative profile sets of all the soil series established in California. The classification of the soils used is given in table 1, and their geographic distribution is indicated in figure 1. The distribution of the average annual rainfall in California (19, p. 3) is also given in the figure.

The descriptions are based on "The Basis of Classification and Key to the Soils of California" by Shaw (17). The Indian soils are from the general vicinity of Allahabad in a region of high annual precipitation and high mean temperature. They were collected by Pendleton and are described more fully in the soil survey reports of the Gwalior, Mandsaur, Ujjain, and Shivpuri areas, India (13).

All of the soils had been preserved in glass jars for at least one year, and in some cases for as long as 15 years, and were air-dry. Each was prepared before use by being passed through a 1 mm. screen, followed by thorough mixing. The Indian soils had been sterilized upon entrance to this country five years previously. Except as indicated, all were surface soils.

THE PLASTIC CONSISTENCY

Plasticity, or the plastic consistency, may be defined as a property of solids by virtue of which they hold their shape permanently under the action of small shearing stresses, but are readily deformed, worked, or molded, without cracking, under somewhat larger pressures. The main part of the definition is according to Bingham (4, p. 216). Mellor (12, p. 354) adds "without cracking" to an essentially similar but independent definition. It is obviously possible to exclude the very sandy soils from those which exhibit plasticity under ordinary conditions to an even moderate degree. Accordingly, the coarse textured soils have been excluded from the group examined.

Atterberg recognized several consistency forms. He recognized the plastic consistency by his ability to roll the soil out into a wire. The moistness at which this just ceases to be possible he named the "Ausrollgrenze," or lower limit of plasticity. He considered the upper limit of plastic consistency, or the "Fliessgrenze," to be that moisture content at which the bottom parts of a V-shaped furrow in a cake of soil, in a small, round-bottomed dish, just flow together when the dish is jarred. Commenting upon the "Fliessgrenze," Atterberg stated (2, p. 37), "This is not a sharp, natural limit. Such a limit probably exists for loams but not for clays. Therefore the position of the limit must be considered as arbitrary." He made no statement of the number or magnitude of the impacts to be used, beyond stating that "the dish must be rapped violently and repeatedly against the palm of the hand" (ibid. 2, p. 37).

From a consideration of Bingham's fundamental conception of the flow of plastic solids having a high degree of homogeneity, when subjected to a shearing force F,

$$Vk = \mu(F-f)$$

where V = volume of flow, k is a proportionality factor, μ is the coefficient of mobility[2] in analogy to the fluidity of liquids, and f represents the force necessary to overcome the internal friction of the material, or that just needed to start the flow. Figure 2 is a flow-shear diagram, after Bingham, for plastic solids possessing different degrees of mobility (as indicated by the slopes of the curves) and of internal friction, or yield values (as indicated by the intercepts on the abscissa).

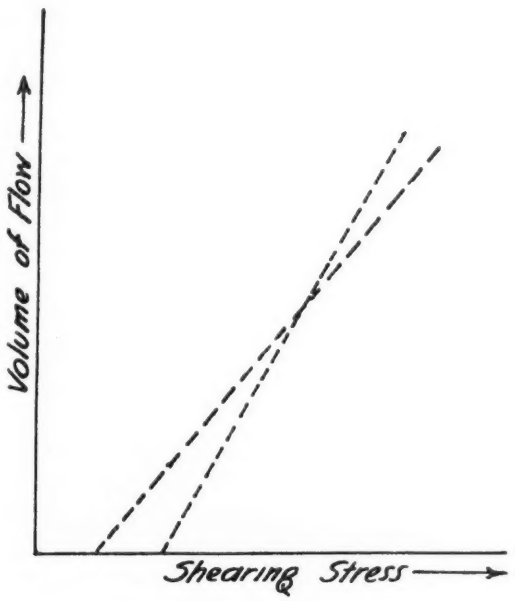

FIG. 2. FLOW-SHEAR DIAGRAM OF PLASTIC SOLIDS
(After Bingham)

By applying this idea to the case of a moist soil at the "Fliessgrenze" of Atterberg, we may write

$$\mu (F - f) = 0$$

which is only true when $F = f$. As F approaches f as a limit the volume of flow approaches zero. It is therefore clear that insofar as Atterberg's conditions of measurement of the "Fliessgrenze" can be reproduced, it must determine the moisture content of the soil at which the shearing force applied just exceeds the internal friction of the material and so causes a very slight flow to take

[2] Bingham defines the mobility of a plastic solid as the reciprocal of its consistency, where consistency is to be thought of as the measure of resistance to rapid deformation.

place. The so-called upper plastic limit must therefore be considered as entirely arbitrary, and dependent for its value upon the strength of the blow struck. Upon increasing either the impact force, or the number of impacts, provided each one always develops a shearing force slightly in excess of the yield value, the upper plastic limit will be lowered to a new moisture content such that the force necessary to overcome the internal friction of the soil is again just below the shearing force transmitted by the blow. It is conceivable that the "Fliessgrenze" may be lowered at least to the "Ausrollgrenze" by increasing the shearing force. Russel and Wehr (15, p. 358) reported instances of practical coincidence of the two values, when determined in the ordinary way for certain soils. It follows that the plasticity number, or plastic range is also entirely arbitrary.

At the "Ausrollgrenze" the soil wire is subjected to a small shearing force when under a pressure slightly in excess of one atmosphere and must be in such a condition of moistness that shearing cannot occur unattended by crumbling.

Considering two different soils, at the same or different moisture content, which have in some way been subjected to the same shearing stress and which in consequence have produced equal amounts of flow,

$$V_1 k = \mu_1(F_1 - f_1)$$

and

$$V_2 k = \mu_2(F_2 - f_2)$$

In this particular case $V_1 = V_2$ ($= V$, the volume of flow) and $F_1 = F_2$ ($= F$, the shearing stress), $\mu_1 =$ the mobility of soil 1, $\mu_2 =$ the mobility of soil 2, $f_1 =$ the internal friction or yield value of soil 1, $f_2 =$ that for soil 2, and so,

$$\mu_1(F - f_1) = \mu_2(F - f_2)$$

and

$$\frac{\mu_1}{\mu_2} = \frac{F - f_2}{F - f_1}.$$

Under these conditions, if $f_1 = f_2$, μ_1 and μ_2 will be equal. Different soils at such moisture contents that the application of equal shearing forces produces equal amounts of flow may be said to be of equal stiffness. That is,

$$\frac{\mu_1 f_1 - \mu_2 f_2}{\mu_1 - \mu_2} = F = \text{a constant},$$

Otherwise stated, at the point of equal stiffness the flow-shear curves of different soils will intersect. It will be seen that this becomes possible for a wide range of soils, provided that their moisture contents may be adjusted. It may be

pictured more readily if a third axis, at right angles to the other two, is added to the coördinate system of figure 2, as in figure 3, to represent moisture content, provided that the system is always viewed in a direction parallel to the third axis. The consistency and yield value will vary with the moisture content.

By controlling the applied force, as for example in an adaptation of Atterberg's method by fixing the number and magnitude of the impacts, and by fixing the volume of flow, a rather simple means may be devised whereby the moisture content can be determined at which soils possess the same degree of

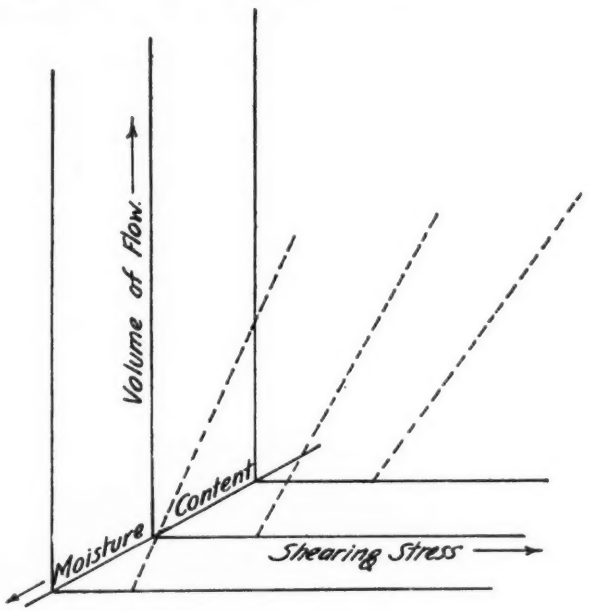

FIG. 3. FLOW-SHEAR DIAGRAM OF A PLASTIC SOLID, THE CONSISTENCY OF WHICH IS MODIFIED BY ITS MOISTURE CONTENT

stiffness. This was attempted in the present work, and the soils were examined as to moisture content when a certain small, fixed amount of flow was obtained under such conditions. It was first necessary to adopt some method for standardization of the impact magnitude. The physical basis for the method used is described in the following.

Dropping an object from a height to a fixed, rigid surface provides a simple way of subjecting it to a relatively large force during the very short time interval of impact. An expression for the magnitude of the force developed in this way may be calculated by consideration of certain elementary mechanical

principles. The momentum of a body falling freely from rest is given, for any instant, by the product of its mass m, and its velocity v,

$$\text{momentum} = mv$$

which may be expressed[3] in terms of force F, and time t,

$$\text{momentum} = Ft$$

Upon striking the rigid surface at the distance a from its starting point the falling body almost immediately comes to rest, then[4]

$$\text{linear impulse} = \text{change in momentum} = -mv$$

The change in momentum is due to an impressed force F', the magnitude of which at the time of impact may be measured by the change in momentum, and is given by

$$F' = \frac{m(v_2 - v_1)}{t'}$$

where v_1 = the initial velocity, v_2 = the final velocity, and t' = the time during which the force acts. In this case

$$F' = -\frac{mv}{t'}.$$

Since $v = \sqrt{2gh}$, for unit mass $F' = -\frac{\sqrt{2gh}}{t'}$. The change in momentum, $-mv$, is brought about in the very small time interval t', so that F' may be of considerable magnitude.

[3] In the case of a freely falling body, neglecting air resistance, the acceleration, α

$$= \frac{d^2s}{dt^2} = g, \text{ the gravity constant}$$

$$\frac{ds}{dt} = gt + v_0 = v, \text{ the velocity at any instant}$$

and

$$s = 1/2\ gt^2 + v_0t + s_0 = \text{the distance fallen at any instant}$$

For a body starting from rest, where distance is measured from the starting point, V_0 and $s_0 = 0$, so that $s = 1/2\ gt^2$ and $v = gt$. If $s = h$, the height, then $h = 1/2\ gt^2$, and $v = \sqrt{2gh}$.

Since $F = m\alpha$, the mass may be expressed as $\frac{F}{g}$ and therefore momentum $= \frac{F}{g}.gt = Ft$.

[4] The linear impulse is given by measurement of the total change in linear momentum, which is obtained by measurement of the change in velocity.

If v_1 = velocity of falling body just before striking the rigid surface, and

v_2 = its velocity just after the blow has been struck,

Since $v_1 = v$ and $v_2 = 0$ cm./sec., then the

$$\text{impulse} = \text{change in momentum} = m(v_2 - v_1) = -mv.$$

EXPERIMENTAL

The procedure which involves these principles and which was finally adopted, follows. A moisture determination is made of the air-dry soil. Sufficient soil to give 20 gm. of water-free material is then weighed out into a small, round, straight-walled and flat-bottomed aluminum dish (50 mm. diameter x 13 mm. high). From the weight of dish and soil a table is previously prepared showing the gross weights corresponding to different moisture percentages over the working range. Water is then added to the soil from a burette and thoroughly mixed into it by manipulation with a stiff, short-bladed spatula. It is usually convenient to perform the initial mixing operation by repeatedly chopping the moistened soil with the edge of a spatula on a glazed tile. The moist soil is pressed in the puddled[5] condition into the aluminum dish, smoothed level at the top, and weighed. It is then quickly provided with a V-shaped ditch having sides sloping at an angle of 45° with the perpendicular. The excavated wedge of soil is removed to a porcelain dish and placed in a humidifier during the dropping process which follows, for return to the soil mass after the ditch has been filled. The ditch should be as clean as possible, smooth-walled, with a right-angled vertex slightly above the bottom of the soil layer, of uniform width and depth each time and situated at the diameter of the dish. The impact used in the production of plastic flow is then provided by letting the dish fall freely and squarely from a height of 30 cm. to a heavy laboratory table. A given amount of flow is considered to have taken place when a succession of equal impacts has been provided, sufficient to fill the ditch. The number of impacts necessary is recorded. The exact moisture content as percentage on the dry basis may be calculated readily from the prepared table of gross weights by interpolation. By repeated additions and incorporations of water, weighings, ditchings, and droppings, data are obtained for the construction of a moisture content—impact curve. The maximum size of a ditch having the sectional form of an isosceles right triangle, vertex down, and in turn the amount of flow, are of course controlled by the depth of soil in the dish.

A ditch having a width at the top of 14 mm. and a depth of 7 mm. proved satisfactory. This gave a volume of about 2.3 cc. In all cases the size of the ditch was kept as nearly constant as possible. The width was controlled by parallel rulings on the smoothed surface with a caliper-like device cut from tin, the actual ditching being done with two razor blades held at an angle of 45° with the soil surface, and a spatula to remove the soil wedge.

Errors will be produced by non-uniformity in moisture distribution, incomplete puddling, construction of an imperfect ditch, high evaporation losses, and erroneous decision as to when the ditch is filled. The method of initial wetting has already been described. Later additions of water are usually more easily incorporated with the already moist cake by running the water on from a

[5] For the present purpose a puddled condition is considered to be one in which the pore space of the soil has been sufficiently reduced that true plastic deformation may take place.

burette, cutting the soil checkerboard fashion with the spatula, and then thoroughly working up the soil. In order that none of the impacts shall be used up in puddling the soil care must be taken to prepare a well-puddled mass in the dish before ditching.

The preparation of a clean ditch of constant size is difficult with the stickier soils at high moisture contents. The loss of water during the dropping was found to be of relatively slight effect except in the drier stages. This was checked by making a moisture determination at the end of the run for each soil. The final water content was found to agree closely with that desired. With care, soil losses due to spattering are slight and seldom exceed 0.1 gm. in 20 gm. of soil.

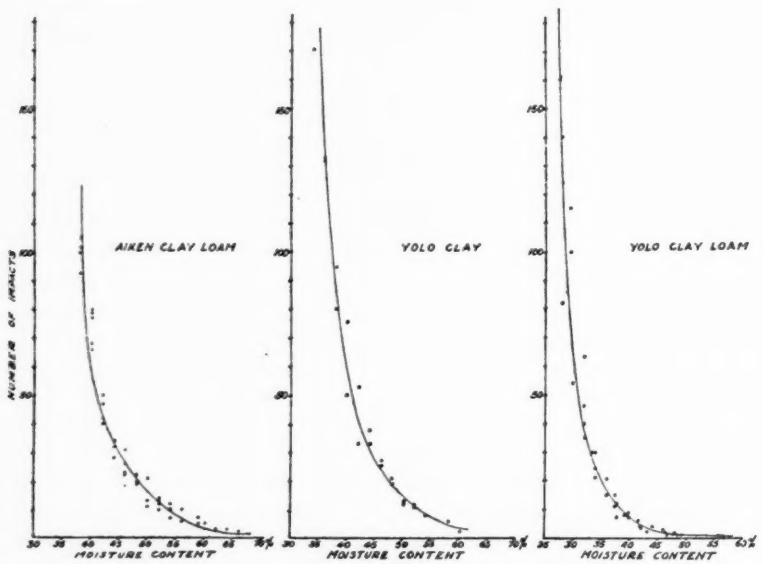

FIG. 4. MOISTURE CONTENT—IMPACT CURVES FOR AIKEN CLAY LOAM, YOLO CLAY, AND YOLO CLAY LOAM

Decision as to when the ditch may be considered filled presents some difficulty. The limit of readjustment of the soil mass after the construction of the ditch and consequent upon the dropping is clearly not reached until the surface of the soil cake is again level, but in a lower position than it originally occupied by an amount corresponding to the volume of the soil wedge excavated. In order to reduce evaporation losses, the ditch was considered to be filled when a number of blows had been delivered sufficient to cause the flattened walls of the ditch to be in practically continuous line (in cross section) with the very slightly sloping surface of the cake on either side of the ditch. This may be

judged by holding a straight edge at right angles to the line of the ditch, and resting vertically upon the soil.

No attempt was made to measure the absolute value of F', a large part of the total force being consumed in various ways other than in the production of plastic flow. The important thing is that for unit mass, or any small element of the total soil mass, F' is reproducible with considerable accuracy, because of its direct proportionality to the square root of the height from which the soil falls, a value which is easily held constant.

When the number of impacts needed to produce a given quantity of flow are plotted against the corresponding soil moisture contents, curves of the power type are obtained. These are plotted for the Aiken clay loam, the Yolo clay, and the Yolo clay loam in figure 4. Several sets of values were obtained for each of these soils. An idea of the possible duplication of the results may be obtained from the position of the experimentally determined points with respect to the smooth curve drawn through them.

The relationship between the number of impacts needed to produce an arbitrary, fixed amount of flow in a certain soil and the water content of the soil, may be examined by assuming that $i = aw^n$, when $\log i = \log a + n \log w$, where i = the number of impacts needed to fill the ditch, w = total percentage of water (dry basis), and a and n are constants. By carefully plotting the experimental values of i and w upon coördinate paper to a logarithmic scale, a straight line is obtained with negative slope. Values for a and n are obtained by graphic solution, when a turns out to be large and positive, and n very much smaller and negative. The significance of the relationship becomes clearer if positive values of n are dealt with, in which case $i = \frac{a}{w^{-(n)}}$. This is the equation for a hyperbolic curve to which both coördinate axes are asymptotes. It will be seen that for a given soil, that is to say for fixed values of a and n, i increases in inverse proportion to some power of w. The curves Yolo clay loam and Yolo clay (fig. 4) are plotted to a logarithmic scale in figure 5. Their equations are

$$\text{Yolo clay,} \qquad i = (7.1 \times 10^{12})\, w^{-5.92}$$
$$\text{Yolo clay loam,} \quad i = (1.3 \times 10^{13})\, w^{-7.68}$$

Impact number—moisture content measurements were made for 50 other soils. The results were plotted to a logarithmic scale and the straight line relationship was found to hold in every case. Figure 5 gives a few of these.

Moisture contents at which they have the same stiffness may readily be determined from a diagram which includes logarithmic graphs of the impact moisture relationships for all of the soils. Such a diagram was prepared, and the moisture contents compared when 100 impacts produced an equal amount of flow in all soils (table 2).

The other determinations reported in table 2 were carried out as follows:

1. Air-dry moisture content. About 5 gm. of soil was heated at 105°C. for 24 hours in glass weighing bottles, the loss in weight being reported as percentage of dry weight of soil.

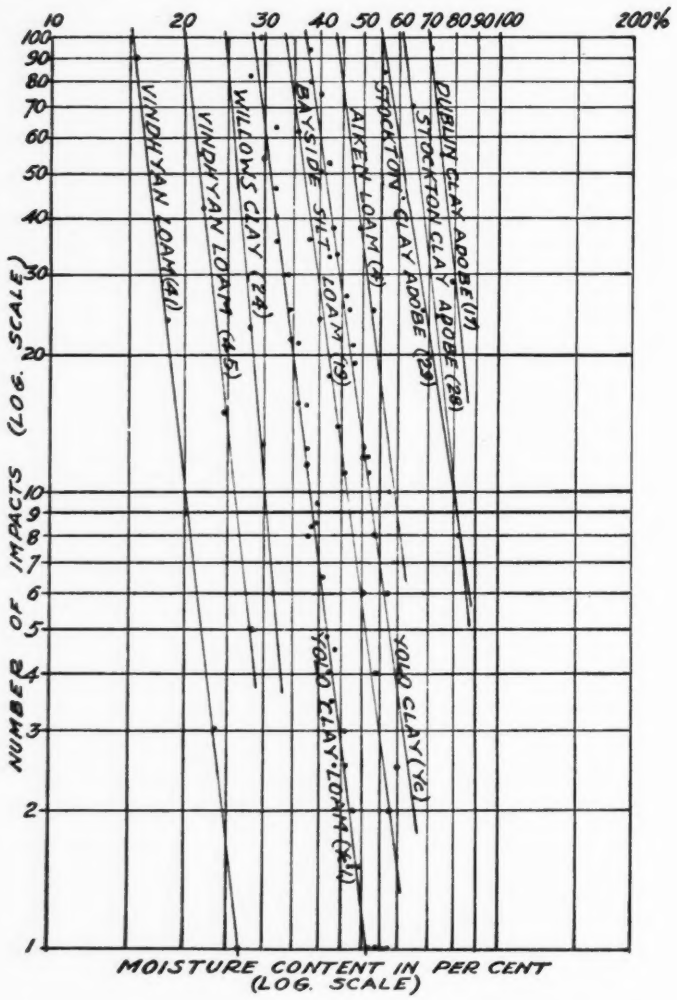

FIG. 5. MOISTURE CONTENT—IMPACT NUMBER CURVES PLOTTED TO A LOGARITHMIC SCALE

2. Moisture equivalent. Thirty grams of air-dry soil were used. The samples in the centrifuge cups were kept saturated in pans of water for 24 hours, drained for 30 minutes, and then placed in the standard centrifuge machine. The machine was brought up to speed

TABLE 2

Soil properties

(Soils are arranged in ascending order of "water content at 100 impacts")

SOIL NUMBER	WATER CONTENT AT 100 IMPACTS	AIR-DRY MOISTURE CONTENT	MOISTURE EQUIVALENT	WATER VAPOR ABSORBED OVER 3.3 PER CENT H_2SO_4	COLLOIDAL CLAY CONTENT	LOSS ON DIGESTION WITH HYDROGEN PEROXIDE	pH
	per cent	*per cent*	*per cent*	*gm. per gm. soil*	*per cent*	*per cent*	
41	15.2	1.02	16.5	.0413	13.8	1.13	6.05
45	19.8	1.85	18.6	.0652	21.7	0.85	6.36
44	21.2	2.02	20.2	.0650	21.7	1.67	6.37
42	21.6	2.07	20.2	.0556	18.5	1.55	5.75
24	24.6	2.31	20.9	.0595	19.8	1.99	6.44
33	27.2	4.74	27.0	.1012	33.4	1.28	8.48
43	29.0	2.57	20.1	.0721	24.0	1.18	7.54
6	29.2	6.18	23.1	.0862	28.7	0.45	6.39
10	29.3	4.67	26.3	.0925	30.8	2.38	6.70
40	31.4	4.02	23.7	.0815	27.2	2.44	6.43
25	32.2	3.57	32.2	.0981	32.7	1.22	6.65
49	33.4	4.31	27.0	.1052	35.1	0.47	7.76
19	33.4	2.75	27.3	.0642	21.4	2.96	5.77
18	35.6	3.73	28.2	.0957	31.9	3.33	6.60
22	36.0	6.42	33.2	.1258	41.9	2.97	7.78
11	37.0	6.05	28.1	.1228	40.9	2.13	6.87
50	39.4	5.47	27.3	.1105	36.8	0.89	7.78
12	43.0	6.82	29.8	.1367	45.6	1.68	6.62
5	43.0	5.39	29.1	.1163	38.8	1.51	8.09
4	43.2	4.80	35.3	.1701	56.7	1.53	5.55
36	43.4	4.22	29.6	.1215	40.5	0.69	7.97
47	44.6	6.92	35.2	.1487	49.6	0.53	7.97
38	46.0	4.53	29.2	.1445	48.2	0.40	8.14
48	46.0	5.99	34.8	.1481	49.4	2.09	7.59
3	46.0	7.74	33.7	.1502	50.1	3.36	6.62
27	47.0	5.52	28.9	.1651	55.0	1.15	6.22
20	47.5	3.12	39.1	.1104	36.8	4.81	5.62
37	47.5	4.50	33.3	.2031	67.7	0.30	7.99
16	49.2	5.59	32.6	.1309	43.6	4.11	6.85
8	49.5	6.04	37.7	.1527	50.2	2.74	7.02
26	49.5	5.99	33.4	.1358	45.3	2.50	7.08
2	51.0	7.54	44.3	.2623	87.4	1.57	7.51
1	51.5	7.16	33.7	.1590	53.0	1.57	7.94
39	52.0	4.95	32.8	.1275	42.5	1.14	8.48
14	52.5	7.73	35.5	.1686	56.2	2.46	6.74
21	53.0	5.56	38.6	.1764	58.8	2.43	9.13
32	54.0	6.86	33.0	.1464	48.8	1.09	6.19
34	55.0	9.31	35.4	.1670	55.7	0.84	8.65
29	55.0	6.53	34.8	.1500	50.0	1.26	5.85
30	55.5	6.68	43.0	.1516	50.5	1.03	6.37
31	55.5	6.82	30.6	.1492	49.7	1.92	5.66
13	56.4	7.82	43.1	.1697	56.6	3.17	6.71
15	58.0	6.13	43.4	.1523	50.8	4.67	6.30
35	58.6	9.91	37.6	.2037	67.9	0.68	8.71
9	61.0	8.13	38.1	.1629	54.3	1.57	7.16
28	61.0	7.90	42.8	.1575	52.5	0.81	7.18
23	63.0	8.96	39.4	.1879	62.6	1.97	6.94
46	64.0	8.47	41.5	.1952	65.1	1.38	8.11
17	70.0	9.67	49.3	.2000	66.7	2.99	6.95
7	71.0	11.5	43.2	.1140	38.0	5.43	7.21

gradually and maintained at between 2,450 and 2,475 r.p.m. for 30 minutes, after which the power was shut off and it was allowed to come to rest without the use of brakes. The soils were then rapidly transferred to covered cans and moisture determinations made in the usual way.

3. Water vapor adsorbed over 3.3 per cent sulfuric acid. The method of W. O. Robinson (14) was followed, with the exception that the soils were not ground to pass a 100-mesh sieve, previous experiments having shown this to be unnecessary. The adsorption was continued in partially evacuated glass desiccators, held at 30°C. for 5 days in a large air thermostat.

4. "Colloidal clay" content. An approximation to this value was obtained by dividing the water vapor adsorbed per gram of soil by 0.3 and multiplying by 100. In connection with some vapor pressure studies under way in this division the validity of the average factor 0.3[6] as a means of calculating the approximate amount of colloidal material in soils was later examined for samples of colloidal clay separated from some other Californian soils. The results are given in table 3. The colloidal matter for the 5 soils was obtained by centrifuging at 35,000 r.p.m. from an aqueous suspension of clay having a maximum particle diameter of 0.001 mm., previously fractionated by sedimentation. No dispersing agent other than water

TABLE 3

Water vapor adsorption by different soil colloids over 3.3 per cent sulfuric acid for 5 days

SOIL NUMBER	NAME OF SOIL	WATER VAPOR ADSORBED PER GM. OF COLLOID
		gm.
321	Aiken (Horizon A)	0.283
322	San Joaquin (Horizon B)	0.298
323	Stockton subsoil	0.307
324	Yolo (Horizon A)	0.317
325	Peat	0.296
Mean.....		0.300

was used and the soils were not triturated. After removal from the centrifuge bowl the colloid was air-dried and gradually brought to constant weight over sulfuric acid. It was then ground to pass through a 0.25-mm. mesh screen and the powdered material used for the adsorption trials. No attempt was made to separate all of the colloid from the soils. There is close agreement in the average water vapor adsorption per gram of colloid with that obtained by Robinson. A narrower range was obtained, but that was to be expected, as only one-seventh as many soil colloids were used.

5. Loss on digestion with hydrogen peroxide. Two grams of oven-dried soil were weighed into a freshly dried 50-cc. Erlenmeyer flask, to the neck of which had been fused a 7-cm. length of glass tubing to act as a reflux condenser, and a shorter, slanting, side-tube to permit insertion of the soil. The flask and contents were next counterpoised against a similar, but empty, flask. Then 5 cc. of water and 10 cc. of 30 per cent hydrogen peroxide were added, the mixture was shaken, allowed to digest for 1 hour without external heating, placed on the steam-bath for 1 to 1½ hours, removed to an oven with the corresponding counterpoise flask, brought to approximate dryness, and then dried for an additional 24 hours, before being cooled and weighed against the same counterpoise cooled in the same way. The loss in weight, after correction was made for the solid residue remaining from the heating and evaporation of 10 cc. of the hydrogen peroxide, was considered as organic matter.

[6] W. O. Robinson obtained a mean value of 0.298 gm. per gm. of soil colloidal matter.

6. Hydrogen-ion concentration. This was determined electrometrically with a Leeds and Northrup Laboratory Type Potentiometer, using a hydrogen electrode and a soil-water mixture in the ratio 1:2.

DISCUSSION

From the relationship $i = aw^n$, which is established in the present work for the higher values of w, it is evident that as the total water content diminishes the number of impacts necessary to produce the prescribed amount of flow increases indefinitely. This relationship is very similar to that obtained by Haines (9) when comparing the extrusion pressure in tons per square inch with the moisture content of certain porcelain clays. Haines experienced difficulty in the case of soils at the lower moisture contents because of interlocking of soil

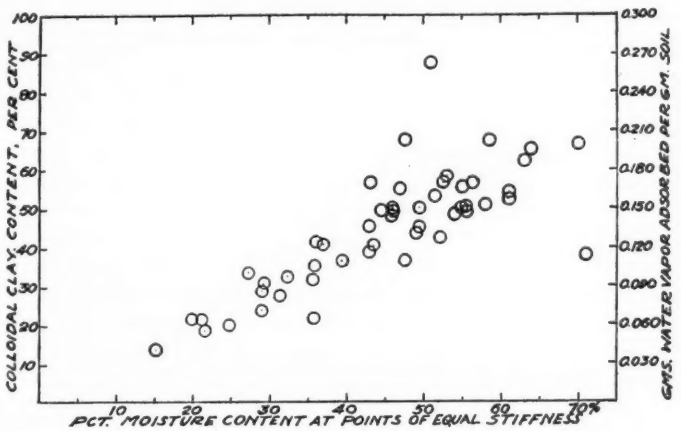

FIG. 6. RELATION BETWEEN COLLOIDAL CLAY CONTENT AND MOISTURE CONTENT AT POINTS OF EQUAL STIFFNESS

Colloidal clay content calculated from the amount of water vapor absorbed over 3.3 per cent sulfuric acid.

particles and squeezing out of water. It is doubtful whether the impact method could be used to examine the relationship for soils in the very dry condition because of the necessity of producing and maintaining a puddled condition, and of applying an impact force in excess of the yield value. Both methods present difficulties when it is desired to explore regions of low moisture content. However, it does appear that fairly heavy soils may be considered as plastics over a wider moisture range than is ordinarily conceded. The increase in plasticity with pressure, for a given moisture content, is well known among ceramic workers (12) and is explained by Haines (8) on the basis of a gradient in pressure of attraction between the clay particle and its water film on passing from the outer to the inner film layers.

The dependence of the moisture content at which one soil is of the same stiffness as another upon the actively water-vapor adsorptive material in the soil, is well indicated by the scatter diagram of figure 6. There is found to be a positive correlation of 0.797 between these two sets of values. According to the theory of plastic flow postulated by Searle (16) for clays, their plasticity depends upon the presence of a lubricating colloidal suspension surrounding the non-plastic particles. The present evidence indicates that the greater the quantity of the colloid in a soil the less perfect the lubrication between the aggregates for a given moisture content, and hence the less mobile the material. Increase in water content is accompanied by a thickening of the water films about the colloidal aggregates and a more ready shearing of the plastic mass. It appears reasonable to suppose that a change is brought about in both the yield value and the mobility, the former being decreased and the latter increased. At high moisture contents, where f is small as compared with the force produced by a single impact, and where the number of impacts and volume of flow are fixed, the soils evidently have very nearly the same mobility. Comparison of the moisture contents at which they have the same stiffness would then become one at which their mobilities were practically the same. The absolute measurement of the colloid content of a soil, if that is possible, and even the definition of the term "colloidal clay," are at the present time admittedly imperfect, and vague, yet from the observations recorded here it seems certain that the moisture content at which the stiffness reaches a certain value depends, for each soil, upon the amount present of a material which has as one of its properties a high adsorptive capacity, a typically colloidal characteristic. No attempt was made in the present work to investigate the nature of the colloidal material, other than by means of the hydrogen electrode, nor of its absorptive capacity for replaceable cations. According to Baver's observations (3) both of these properties may be expected to exert appreciable influence upon soils in the plastic state. Their effect upon the present group of soils, or upon other similar ones, is reserved for later investigation.

The scatter diagrams (figs. 7 and 8) obtained by plotting the air-dry moisture content and the moisture equivalent (table 2, columns 3 and 4) respectively, against the moisture content of the soils at points of equal stiffness (table 2, column 2) indicate the same close relationship ($r_{2.3} = +0.855$ and $r_{2.4} = +0.901$) to exist in these cases as is true for the correlation already given between the amount of water-vapor adsorbed and the moisture content of the soils at points of equal stiffness. The significance of the three coefficients may be examined most readily by reference to a table by Fisher (7, p. 176) in which are given values of the correlation coefficient for different levels of significance when different numbers of observations are made. From this table it is found that the probability that such correlations as have been obtained would arise by random sampling of an uncorrelated population is exceedingly low; namely, much less than .01 in each case. It is evident that a close relationship would be found to exist between these values at any of the comparable points upon

the logarithmic curves, because of the similarity in slope of the latter. The results of the experiment suggest that a scale of relative stiffness could be planned in which stiffness is measured by the number of standard impacts

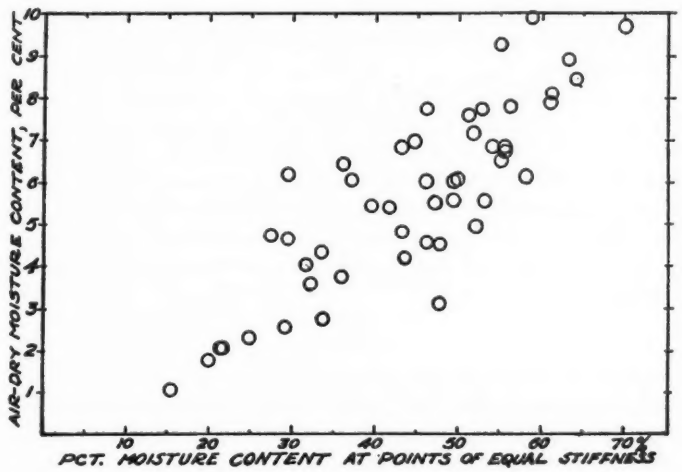

FIG. 7. RELATION BETWEEN THE AIR-DRY MOISTURE CONTENT AND MOISTURE CONTENT AT POINTS OF EQUAL STIFFNESS

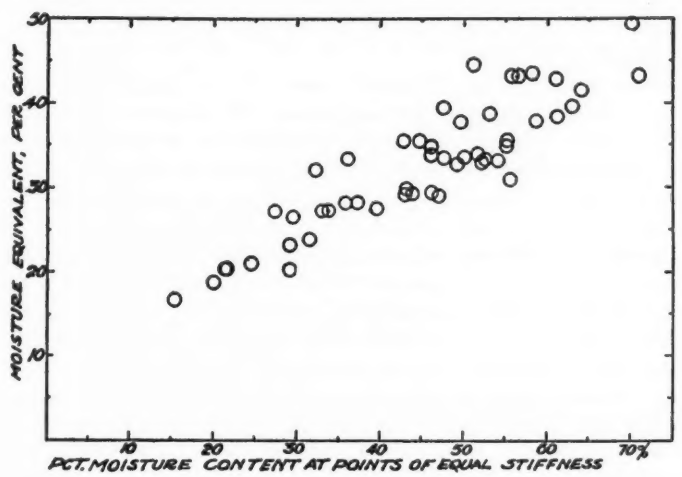

FIG. 8. RELATION BETWEEN THE MOISTURE EQUIVALENT AND MOISTURE CONTENT AT POINTS OF EQUAL STIFFNESS

needed to fill a ditch of standard size. In the present case, for example, the relative stiffness of the soils whose impact moisture curves are drawn in figure 5, may be obtained for any desired moisture content by a vertical section through the graph. It must be borne in mind, however, that extrapolation of the curves into regions of the lower values of w is not permissible beyond a point where the effective force of the impact is only just equal to the internal friction of the soil.

No relation is apparent between the order of arrangement of the soils at moistnesses of equal stiffness and their hydrogen peroxide decomposible organic matter. It must be concluded that for the soils examined, which are of rather low organic matter content, the latter simply behaves as an additional amount of colloidal material. Side-effects may exist for higher contents of organic matter. No distinct correlation was apparent between soil reaction and stiffness. The importance of the amount of colloidal material is thus further emphasized.

SUMMARY

A number of soils of different origin, family, stage of development, group, and series, and developed under different climatic conditions, were examined for several physical and a few chemical properties with regard to the relation existing between these properties and the behavior of the soils in the plastic state.

The soils used had moisture equivalents ranging from 16 to 51 per cent, colloidal clay contents, as measured by the water vapor adsorption method, from 15 to 64 per cent, pH values from 5.55 to 9.13, and were predominantly low in organic matter as measured by digestion with 30 per cent hydrogen peroxide.

By closer attention to, and greater control of, the details of the method originally devised by Atterberg for measuring the moisture content of soils at the so-called "Fliessgrenze," and by consideration of the laws of flow of plastic solids developed by Bingham, a simple method was devised whereby the moisture content of soils may be compared at states of equal stiffness. Equal stiffness is defined as that consistency at which the application of an equal force produces in different soils an equal amount of flow. The method is described, and involves dropping a flat-bottomed, straight-sided, metal container, holding the plastic mass of soil of known moisture content, from a fixed height to a fixed, flat surface, until a ditch, previously excavated in the soil, is filled. By determining the number of impacts needed to fill the ditch at different moisture contents of the soil, and by graphically plotting the results, hyperbolic curves were obtained in all cases. There was found to be a close positive correlation between the air-dry moisture content, the moisture equivalent, and the colloidal clay content, respectively, and the soil moisture content at states of equal stiffness. This proved true for all degrees of stiffness, and

indicates that large quantities of water are bound by the soil colloidal material and that lubrication of the plastic mass is delayed when much colloidal matter is present.

The effect of natural soil organic matter, among the soils used, appeared no different from that of inorganic colloidal material. The hydrogen-ion concentration of the soils was without any distinct effect upon the amount of moisture needed to produce a given stiffness.

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SOME CHEMICAL PHASES OF SUBMERGED SOIL CONDITIONS

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In this paper the results of some investigations on the chemistry of submerged soils and submerged soil solutions are presented with the hope of throwing some light on the failure of such soils to grow satisfactory crops. Hitherto this failure has popularly been ascribed to lack of aeration, lack of nitrites, and other conditions caused by the lack of oxygen.

Preliminary laboratory experiments showed several specific causes of the toxicity of submerged soil solutions. These solutions differ markedly from aerated soil solutions in that they contain relatively large quantities of manganese and iron. Hydrogen sulfide and other poisonous gases are developed, and in cases where the soil is long submerged, the soluble bases are largely exhausted.

In the course of the investigation on the composition of submerged soil solutions, it was found that considerable gas was given off by submerged soils. This phase of the problem was studied, as it is closely allied to the composition of the solution and of the residual soils.

PRELIMINARY WORK

A corn field on Congaree silt loam near Colvin Run, Virginia, contained a poorly drained spot which was under water several times after the corn had reached a growth of about six inches. During most of the growing seasons there was little rain and the poorly drained spot was dry on the surface, but the corn did not grow on this spot. Samples of soil solution were taken in the middle of the poorly drained spot and also a few yards away, where corn grew exceptionally well. Qualitative examination showed an unusually large quantity of soluble manganaese and iron and the water extracts of the soils were analyzed quantitatively for these elements. The results are given in table 1.

The data of table 1 show that the concentration of iron and manganese in the subsoil solution of the poorly drained spot is relatively very high. The subsoil solution of this spot is higher in manganese and iron than the surface soil solution and very much higher than the solution of the fertile adjoining spot. The water table of the poorly drained spot was about two feet below the surface and

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the sample taken from the subsoil was submerged in water. There is abundant evidence in the literature that the concentrations for iron and manganese shown are sufficiently high to cause toxicity.

IRON AND MANGANESE IN BOG WATERS

The high iron and manganese content of the poorly drained spot of Congaree silt loam is a sufficient cause for its toxicity, and naturally leads to the examination of bog waters and poorly drained soil solution to see whether the observed condition is general. Accordingly, samples of solutions were taken from a few

TABLE 1

Comparison of iron and manganese in the water extract of well-drained and poorly drained spots of Congaree Silt Loam, Colvin Run, Va.

DEPTH OF SOIL	DRAINAGE	IRON AS Fe_2O_3	MANGANESE AS MnO
inches		*p.p.m.*	*p.p.m.*
0–7	Poor	1.4	2.0
40	Poor	17.0	28.0
0–7	Good	0.9	2.1
40	Good	2.1	2.2

TABLE 2

Iron and manganese in certain bog waters

LOCATION	Fe_2O_3	MnO
	p.p.m.	*p.p.m.*
Quarry in Falls Church, Va:		
Surface	1.6	1.0
18 inches deep	5.6	1.4
36 inches deep; in mud	8.7	4.2
Greenway Downs, Falls Church, Va.:		
About 48 inches deep; in mud	41.2	7.1
Vienna, Va.:		
About 4½ feet deep; in mud	2.9	4.3
Arlington Brick Yards, Va.:		
Surface	1.3	0.8
24 inches deep; in mud	2.1	1.1

nearby bogs in Virginia. There was considerable flow from two of the bogs, and samples were taken from the surface and also just beneath the mud at the bottom. The process of taking the solution was simple. The solution was filtered through a Pasteur-Chamberland tube. This tube was connected by a long rubber tube to the sample bottle which was exhausted by a hand pump. The first liter was discarded in order to get a representative sample.

The Vienna bog is an old natural one. The others are artificial and the bog near Falls Church is of recent origin. The results of the analysis are given in table 2.

Table 2 shows very variable quantities of iron and manganese. Both increase with depth where samples were taken at different depths. There is probably a surface dilution with rain and drainage water from better drained spots. The surface of the bog in Greenway Downs showed the peculiar iridescent film characteristic of chalybeate waters when they come in contact with air. Such waters contain ferrous carbonate and generally manganous carbonate held in solution by carbon dioxide. The ferrous iron at the surface is oxidized and precipitated as a thin film. The deep samples from the Arlington Brick Yards and from Vienna are not especially high in manganese and iron, presumably because these elements have been leached out. The deep sample from the bog near the Falls Church Quarry is higher in manganese and iron, as might be expected from the fact that the bog was of recent origin and probably contains considerable extractable manganese.

TABLE 3

Variation of solubility of manganese and iron with time of extraction

Kelley and McGeorge

SOIL NUMBER	DURATION OF EXTRACTION	MANGANESE AS MnO
		p.p.m.
313	1 hour	2.37
	24 hours	2.37
	7 days	14.1
314	1 hour	2.33
	24 hours	4.75
	7 days	11.9
319	1 hour	1.41
	24 hours	4.28
	7 days	7.07

It appears from these results that bog waters vary considerably in their content of manganese and iron. The quantities of these elements in river and well waters are interesting in this connection. Clarke (3) cities analyses of the Potomac, Mississippi, and James Rivers. The manganese content of these rivers varies from 0.05 to 0.22 p.p.m. of manganous oxide. The water from five wells in Falls Church, Virginia, contained from a trace to 0.15 p.p.m. of manganous oxide and from 0.24 to 1.3 p.p.m. of iron calculated as ferric oxide. Bog waters are therefore considerably higher in manganese and iron than are ordinary drainage waters.

IRON AND MANGANESE IN SUBMERGED SOIL SOLUTIONS

From the preceding data it is reasonable to expect that solutions of submerged soils will be high in iron and manganese. Kelley and McGeorge (18) have shown that the solubility of manganese in Hawaiian soils increases with time of contact with water. The soils they used were shaken with five parts of water in a stoppered bottle. Their results are given in table 3.

TABLE 4

Development of soluble iron and manganese with time of submergence

SOIL SERIES	DEPTH	TOTAL MnO IN SOIL	SOLUBLE MnO 1 DAY	SOLUBLE Fe_3O_4 1 DAY	SOLUBLE MnO 8 DAYS	SOLUBLE Fe_3O_4 8 DAYS	SOLUBLE MnO 22 DAYS	SOLUBLE Fe_3O_4 22 DAYS	SOLUBLE MnO 60 DAYS	SOLUBLE Fe_3O_4 60 DAYS	SOLUBLE MnO 80 DAYS
	inches	*per cent*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Cahaba fine sandy loam, Georgia	0–12	.065	0.8	...	1.4	....	....	....	....	....	....
Cahaba fine sandy loam, Georgia	12–36	.024	None	...	None	....	....	....	....	....	....
Cahaba very fine sandy loam, Louisiana	0–12	.066	0.7	...	4.8	....	....	....	....	....	....
Cahaba very fine sandy loam, Louisiana	12–36	.079	Trace	...	0.1	....	....	....	....	....	....
Bermudian silt loam	0–8	.089	0.9	0.8	14.7	10.7	....	....	....	....	....
Carrington loam, Iowa	0–12	.057	0.5	0.4	1.9	4.3	4.4	20.0	34.0	40.0	....
Cecil clay loam, Georgia	0–9	.147	0.3	0.3	3.0	1.6	3.6	1.0	3.7	1.3	....
Chester loam, Virginia	0–7	.234	1.6	0.5	4.4	2.9	4.0	14.0	36.8	2.3	....
Chester loam, Maryland	0–8	.087	0.4	0.4	4.0	1.6	6.0	2.0	28.3	8.7	....
Clarkesville silt loam, Tennessee	0–10	.372	1.2	Trace	11.4	1.0	21.6	4.0	44.4	2.8	....
Greenville sandy loam, Georgia	0–10	.030	0.4	...	0.8	....	....	....	....	....	....
Greenville sandy loam, Georgia	10–36	.016	0.2	...	0.3	....	....	....	....	....	....
Hagerstown loam, Maryland	0–8	.108	1.6	Trace	1.0	1.2	4.0	7.0	12.0	5.3	....
Huntington loam, Maryland	0–8	.263	3.7	1.1	24.2	15.6	36.4	50.0	50.0	33.0	....
Louisa loam, Virginia	0–7	.186	Trace	...	1.8	....	....	....	....	....	6.4
Manor loam, Virginia	0–7	.142	2.9	...	26.0	....	....	....	....	....	25.2
Manor loam, Maryland	0–7	.112	1.7	...	9.6	5.0	22.8	6.0	14.3	5.8	....
Memphis silt loam, Mississippi	0–6	.027	0.3	...	1.2	....	....	....	....	....	....
Memphis silt loam, Mississippi	6–36	.067	None	...	None	....	....	....	....	....	....
Memphis silt loam, smooth phase, Mississippi	0–6	.036	0.2	...	1.3	....	....	....	....	....	....
Memphis silt loam, smooth phase, Mississippi	6–12	.052	None	...	Trace	....	....	....	....	....	....
Norfolk sand, South Carolina	0–8	.028	0.4	...	2.1	....	....	....	....	....	....
Norfolk sand, South Carolina	8–36	.005	0.2	...	1.9	....	....	....	....	....	....
Norfolk fine sandy loam, Georgia	0–10	.020	Trace	...	1.1	....	....	....	....	....	....
Norfolk fine sandy loam, Georgia	10–36	.007	Trace	...	Trace	....	....	....	....	....	....

Norfolk fine sandy loam, Georgia	0–16	.007	Trace	...	0.9	....	....	....	....	....	....
Norfolk fine sandy loam, Georgia	16–36	.003	Trace	...	None	....	....	....	....	....	....
Orangeburg fine sandy loam, Mississippi	0–10	.185	0.4	...	2.4	1.0	4.4	12.0	....	....	....
Orangeburg fine sandy loam, Mississippi	10–36	.595	Trace	0.6	0.5	0.6	0.6	1.0	0.5	0.8	....
Orangeburg sand, Georgia	0–10	.016	0.3	...	1.4	....	....	....	....	....	....
Orangeburg sand, Georgia	10–36	.011	0.3	...	1.2	....	....	....	....	....	....
Orangeburg sandy loam, Georgia	0–12	.054	0.4	...	1.6	....	....	....	....	....	....
Orangeburg sandy loam, Georgia Penn loam, Virginia	12–36	.027	0.2	...	0.7	....	....	....	....	....	....
		.188	2.0	...	13.8	....	....	....	....	....	33.6
Penn silt loam, Virginia		.042	1.2	...	9.7	....	....	....	....	....	64.4
Ruston fine sandy loam, Louisiana	0–6	.017	—	...	0.8	....	....	....	....	....	....
Sassafras loam, District of Columbia	0–7	.043	1.0	...	10.1	....	....	....	....	....	12.5
Tifton fine sandy loam, Georgia	0–12	.026	0.3	...	1.0	....	....	....	....	....	....
Tifton fine sandy loam, Georgia	12–36	.006	Trace	...	0.3	....	....	....	....	....	....
Wabash silt loam, Nebraska		.052	—	1.0	2.0	1.6	3.3	6.0	7.0	2.1	....

Table 3 shows a steady increase in the quantity of manganese dissolved with time of contact and a comparatively large content of manganese. The soils studied by Kelley and McGeorge were very high in alumina, iron, and manganese, differing considerably in this respect from American soils.

Experiments somewhat similar to those of Kelley and McGeorge have been carried out with American soils. For this purpose about 500 gm. of soil was placed in a bottle with about 2,500 cc. of water and the bottle stoppered. The bottles were shaken thoroughly once a day and samples of the solution were drawn at certain intervals, care being taken to admit as little air as possible. The samples were analyzed for iron and manganese. The results are given in table 4. The third column gives the total manganese in the soil and the remaining columns give the manganese and iron content of the solution in parts per million.

Table 4 shows that the solubility of manganese increases with time of submergence in all cases where there was any appreciable quantity of manganese in the soil. In general, the increase in the solubility of manganese with time of submergence, is greatest when there is an abundance of organic matter in the soil. Subsoils show very little initial solubility of manganese and the solubility does not increase much with time. Whether this is because of the form in which the manganese occurs or of the lack of suitable organic matter, is not known. In general, the subsoils are low in manganese, but one subsoil, that of the Orangeburg fine sandy loam, contained the largest quantity of manganese of any of the samples. The organic matter in this subsoil was but 0.77 per cent. The quantity of manganese made soluble is not proportional to the total quantity present, though, in general, soils low in total manganese show a low solubility. An exception to this generality is the Penn silt loam with only 0.043 per cent manganous oxide, which yielded a solution containing 64.4 p.p.m. In this soil three quarters of the manganese was made soluble. The maximum solubility for manganese was probably not reached in these experiments. The sample of Manor loam from Virginia which showed a solubility of 25.2 p.p.m. of manganese oxide after 80 days, yielded 60 p.p.m. after standing 10 years in a stoppered bottle.

The solubility of the iron also increases with time, but passes through a maximum with some soils. This may be due to the oxidation of ferrous bicarbonate and consequent precipitation of ferric hydroxide by the admission of a small quantity of air when the samples were taken. This oidation takes place rather rapidly, for the submerged soil solutions generally showed the characteristic film of chalybeate waters after standing in the laboratory a few hours. It is possible that the precipitation of iron may be caused by the development of ammonia which, according to Subramanyan (32, 33), somtimes forms under submerged soil conditions. The sample of Manor loam from Virginia which had been standing in a stoppered bottle for 10 years contained only 1 p.p.m. of iron calculated as ferric oxide. There was a heavy precipitate of iron oxide in the solution just above the surface of the submerged soil. The Huntington

loam, Chester loam from Virginia, Hagerstown loam, Wabash silt loam, and Clarkesville silt loam show a decrease in the soluble iron from the 22- to the 60-day period. Iron is evidently precipitated more readily from chalybeate solutions than is manganese. This is in agreement with the observations of Fresenius (3, p. 532).

GENERAL COMPOSITION OF SUBMERGED SOIL SOLUTIONS

In the previous experiments it was noted that some of the submerged soil solutions contained comparatively large quantities of calcium and magnesium. The determination of these elements in submerged soil solutions has little bearing on the toxicity of submerged soils. If, however, the solubility of calcium, magnesium, and other elements is greatly increased by submerged soil conditions, these elements may be dissolved to such an extent as to render the soils unproductive. Accordingly a number of the submerged solutions were analyzed for silica, alumina, iron, manganese, lime, magnesia, potash, and soda. The corresponding analysis of the fresh solutions were also made. The analyses are given in table 5.

Table 5 shows a great increase in the solubility of lime and mangesia as well as an increase in iron and manganese, which has been noted before. Silica and presumably alumina remain much the same as in the beginning, except that the solubility of the silica is somewhat increased in the soils containing much organic matter. Soils low in organic matter such as the Cecil and Orangeburg show very little actual increase in any of the constituents.

Potash and soda show a considerable actual increase in all cases. There is undoubtedly some soil mineral decomposition going on during the long time of submergence. The great increase of the lime, magnesia, iron, and manganese, due mainly to the presence of carbon dioxide, probably causes base exchange processes to be operative. The concentrations of potash and soda would be increased by this cause.

According to the figures given, there is but little increase in the solubility of alumina with time of submergence. Stoklasa (31) reports that a specimen of marsh water contained 273 p.p.m. of alumina. This speciment must have been very acid, for Magistad (22) found that a pH of 3.96 was required to hold 200 parts of alumina in solution. The pH of the solutions examined was not less than 5.0, which is capable of holding only about 2 p.p.m. of alumina in solution. The presence of other soluble material tends to lower rather than to raise the solubility of the alumina, according to Joffe and McLean (15). It is apparent, therefore, that the solubility of alumina is not increased by submerged soil conditions.

The solubility of lime is so high under submerged soil conditions that such a soil would soon lose all the lime it contained, provided, of course, that there was movement of the solution or movement of the lime by diffusion. From a comparison of the composition of the solutions given in table 5 with the composition of the soils given in a previous bulletin (26) it can be seen that soils

TABLE 5

Increase in solubility of various elements with time of submergence

SOILS AND LOCATION	DEPTH	ORGANIC MATTER	IN CONTACT WITH SOLUTION 18 HOURS							SUBMERGED 60 DAYS							
			SiO_2	R_2O_3	MnO	CaO	MgO	K_2O	Na_2O	SiO_2	Al_2O_3	Fe_2O_3	MnO	CaO	MgO	K_2O	Na_2O
	inches	*per cent*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Carrington loam, Iowa	0–12	3.94	9.6	1.7	0.5	17.5	4.0	1.7	6.0	26.8	...	40.0	34.0	147.0	40.0	10.0	18.0
Cecil clay loam, Georgia	0–9	0.56	4.3	0.8	0.3	5.2	0.9	0.7	0.8	5.4	...	1.3	3.7	11.0	3.0	3.2	3.6
Chester loam, Virginia	0–7	1.68	4.1	1.1	1.6	18.5	6.7	1.1	2.1	6.6	...	2.3	36.8	42.8	4.0	12.0	27.1
Chester loam, Maryland	0–8	1.62	3.9	1.2	0.4	14.1	1.2	1.2	2.0	8.0	...	8.7	28.3	81.0	8.0	8.2	14.7
Clarkesville silt loam, Kentucky	0–10	0.96	3.3	1.2	1.2	16.1	6.1	1.2	2.6	5.0	...	2.8	44.4	232.4	14.5	22.2	19.2
Hagerstown loam, Maryland	0–8	1.48	4.0	0.9	1.6	25.7	6.1	2.4	2.1	10.5	2.1	14.0	1.2	88.5	24.5	13.7	21.7
Huntington loam, Maryland	0–8	3.88	...	1.1	3.7	....	...	...	...	26.4	...	33.0	50.0	52.0	28.1	6.3	12.1
Manor loam, Maryland	0–7	1.68	3.7	1.1	1.7	13.2	5.8	3.1	2.2	9.0	1.1	5.8	14.3	40.0	7.5	10.4	25.2
Marshall silt loam, Nebraska	0–14	4.10	13.3	4.1	...	12.6	5.3	4.3	8.9	29.3	3.0	38.1	31.2	169.0	52.0	16.3	21.7
Orangeburg fine sandy loam, Mississippi	0–10	0.77	5.1	1.9	0.4	2.1	1.0	3.6	5.0	10.0	1.0	7.8	14.5	10.5	2.0	7.1	9.3
Wabash silt loam, Nebraska	0–15	3.03	8.2	1.8	...	5.6	2.0	1.3	6.5	31.5	0.8	2.1	7.1	22.0	6.0	8.9	15.4

long submerged would contain little but silica and alumina. This depletion of essential elements is given by Hilgard as a prominent cause of the unproductiveness of poorly drained soils.

EFFECT OF CARBON DIOXIDE ON THE SOLUBILITY OF SUBMERGED SOIL CONSTITUENTS

It will be shown later that gases formed from submerged soils contain carbon dioxide. Solutions saturated with this gas have the property of dissolving large quantities of lime and magnesia, as is well known. If a flask partially filled with some of the more concentrated submerged soil solutions is evacuated, precipitation of manganese, iron, calcium, and magnesium takes place. Those elements are evidently held in solution by carbon dioxide. It is self-evident that the solubility of lime and magnesia in soils would be increased

TABLE 6

Influence of carbon dioxide on the solubility of manganese

SOILS AND LOCATION	MnO DISSOLVED IN 24 HOURS	
	Distilled H_2O	CO_2-saturated H_2O
	p.p.m.	*p.p.m.*
Bermudian silt loam, Virginia	0.9	15.0
Louisa loam, Virginia	0.1	13.9
Manor loam, Virginia	2.9	28.9
Penn loam, Virginia	2.0	23.8
Penn silt loam, Virginia	1.2	3.3
Sassafras loam, District of Columbia	1.0	3.6

by bubbling carbon dioxide through the soil. Presumably the solubility of iron and manganese would be likewise increased. Some experiments were carried out in this connection. A slow stream of carbon dioxide was bubbled through a one to five mixture of soil and water for 24 hours. Manganese was determined in these solutions and also in solutions of the same soils in the absence of carbon dioxide. The results are given in table 6.

Table 6 shows that the solubility of manganese is greatly increased by the presence of carbon dioxide. Several factors are operative here. The reaction is made more acid, calcium and magnesium go into solution as bicarbonates, and a greater exchange of bases takes place. The solution being saturated with carbon dioxide, any manganous or ferrous compounds formed would be dissolved by the carbon dioxide. The solubility of iron is presumably increased by the presence of carbon dioxide, for there is every reason to believe it would act like manganese in this respect.

INFLUENCE OF AIR VOIDS ON THE DEVELOPMENT OF SOLUBLE IRON AND MANGANESE

In the experiments on the development of soluble iron and manganese, (table 4) some air was let into the bottles when samples of the solution were taken. It seemed possible that the oxygen of the air admitted would have some effect on the solubility of the iron and manganese, though the volume of air was comparatively small. To test this point, three soils were submerged in such a manner that nitrogen instead of air replaced the withdrawn solution. Samples were withdrawn at an interval of 30 days. The iron and manganese contents of the resulting solutions are given in table 7.

It is apparent that the introduction of such quantities of air are not significant but it is not to be concluded that soil solutions are independent of the presence or absence of air. When solutions from submerged soils are allowed to stand in an open beaker they become cloudy, and a reddish brown precipitate soon forms. The same precipitate is formed when air is slowly bubbled

TABLE 7

Comparison of iron and manganese solubilities in stoppered bottles with air and nitrogen voids

SOIL AND LOCATION	30 DAYS DURATION, 1:5			
	Nitrogen-filled voids		Air-filled voids	
	Fe_2O_3	MnO	Fe_2O_3	MnO
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
Carrington loam, Iowa	34	8.0	31	9.6
Chester loam, Virginia	18	11.6	16	10.2
Hagerstown loam, Maryland	14	6.6	12	7.6

through the solution. The iron is first deposited. Long continued contact with air produces a separation of a portion of the manganese.

THE REACTION OF SUBMERGED SOIL SOLUTIONS

The reaction of submerged soil solutions is of interest in connection with the toxicity of such solutions. It is also important as a possible cause of the greatly increased solubility of the bases, particularly of ferrous iron and manganese. The solubility of the last two named elements is greatly increased with increasing hydrogen-ion concentrations of soil solutions.

It is commonly supposed that submerged soil solutions are more acid than aerated solutions of the same soil. On the contrary, Gillespie (8) and Subramanyan (32) have found that submerged soil solutions are less acid than aerated solutions. Subramanyan found that the nitrogen compounds in the rice paddy fields rapidly develop ammonia, which decreases the hydrogen-ion concentrations.

The hydrogen-ion concentration of a number of soils and solutions resulting from 60 days submergence has been measured. In all cases, as shown in table 9, the gas in contact with the solutions contained carbon dioxide. The apparatus used to measure the hydrogen-ion concentrations was of the bubbling cell type and is not particularly well adapted for this purpose, since the dissolved carbon dioxide is rapidly displaced by the hydrogen. The readings were therefore taken as quickly as possible and another reading was taken after the hydrogen had been bubbled through the solution for 60 minutes. The results are given in table 8.

Table 8 shows that the reading when first taken was usually about pH 6. The readings were very variable and rose rapidly at first. The Cecil and Orangeburg series behaved differently from the others. There was very little

TABLE 8

Reaction of certain soils and submerged soil solutions

SOILS AND LOCALITY	SOIL	SOLUTION	
		First reading	After 60 minutes bubbling
	pH	*pH*	*pH*
Carrington loam, Iowa	5.18	7.2	7.60
Cecil clay loam, Georgia	5.44	6.0	6.22
Chester loam, Virginia	6.33	6.5	8.10
Chester loam, Maryland	6.50	6.6	8.00
Clarkesville silt loam, Tennessee	6.20	5.5	7.93
Hagerstown loam, Maryland	7.58	6.2	8.53
Huntington loam, Maryland	5.83	6.6	8.36
Manor loam, Maryland	5.80	6.0	7.93
Orangeburg fine sandy loam, surface, Mississippi	5.12	4.5	4.48
Orangeburg fine sandy loam, subsoil, Mississippi	4.70	4.7	4.70
Wabash silt loam, Nebraska	....	6.0	7.50

change in the reachings with time in these two soils and the Orangeburg surface changed to a lower rather than a higher reading with time. Table 8 shows that the Cecil and Orangeburg soils developed very little soluble constitutents with submergence, a property doubtless connected with this behavior.

After hydrogen had been passed through the solution for about an hour the majority of the readings became constant (for any one soil) at a pH value varying between 7.5 and 8.5. This point is probably governed by the reaction of the carbonates precipitated from the solution by the displacement of the dissolved carbon dioxide by hydrogen.

The foregoing values were taken after the solution had been submerged 60 days. Since Subramanyan (32, 33) has shown that submerged soils give off ammonia at some stages of submergence, it is possible that these solutions might have been alkaline at some earlier stage. It would be difficult to recon-

cile this with the observed continually increasing concentration of iron and manganese in solution. It would seem that the pH would be governed by the carbon dioxide evolved and this was sufficient to nearly saturate the solution each time when tested.

GASES FROM SUBMERGED SOIL

In nearly all the experiments it was noticed that gas was given off by the submerged soil mass. The solutions are therefore saturated with these gases and their composition may be expected to have an influence upon the toxicity of the solutions. A study of the composition of these gases was therefore undertaken.

The gases from submerged soils may be expected to resemble the gas sometimes arising from marshes. This "marsh gas" consists mainly of methane with a small quantity of carbon dioxide, and occasionally hydrogen is reported.

Gases from submerged soils owe their origin to the decomposition of organic matter by anerobic bacteria. Omelianski (25) has identified a number of such soil bacteria. These microörganisms produce mainly methane and hydrogen and in some cases small quantities of hydrogen sulfide and mercaptans.

Harrison and Subramania Ayer (10) determined the composition of a large number of gas samples from rice paddy fields. They found that the gas dislodged by poking the soil in a submerged and recently transplanted rice field consisted largely of methane with small quantities of hydrogen, carbon dioxide, nitrogen, and oxygen. In the gases arising normally from the surface of a growing rice field, methane and hydrogen disappear and the gas consists of nitrogen, carbon dioxide, and oxygen. They account for the difference of composition of the gases developed by assuming that the blue-green algae growing on the paddy fields decompose the marsh gas and convert it into carbon dioxide and water and the hydrogen into water.

The gases were generated and collected in the following manner. Four hundred or five hundred grams of soil were placed in a 2½-liter bottle. The bottle was entirely filled with water, making about a one to five mixture. After this mixture had stood two or three days the fine bubbles and floating organic matter collecting on the top were floated off by water and the bottle was stoppered and connected to an inverted flask or endiometer entirely filled with water. In this way water displaced the air in the entire apparatus. The connection was so made that the arising gas displaced the water in the flask or endiometer. The end of the outlet tube was placed several inches under water so there was no opportunity for the air to suck back into the collecting chamber. Under these conditions gas commenced to appear after from two weeks to two months. The rates of gas evolution varied greatly with different soils and at different times with the same soil. In one case 1,000 gm. of soil yielded 250 cc. gas daily; with another soil about 5 cc. was given off in the same time. In general, the gas evolution was more rapid at higher than at lower

temperatures. In all cases gas was eventually produced. No attempt was made to effect microbiological control. The analyses of the gases obtained are given in table 9.

The analyses given in table 9 show the gases to be mainly methane, generally with some hydrogen, nitrogen, carbon dioxide, oxygen, and in some cases carbon monoxide. Qualitative examination showed the presence of a small quantity of sulphur in the gases.

The observations of Harrison and Subramania Ayer (10) are confirmed with respect to the disappearance of hydrogen and the formation of a large quantity of nitrogen in submerged soils where blue-green algae are present. It will be noted that no hydrogen was given off by the Carrington, Hagerstown, Cecil, and one sample of the Wabash soils. The solutions from these soils supported a

TABLE 9

Composition of gases from submerged soils

	METHANE	HYDROGEN	NITROGEN	CARBON DIOXIDE	CARBON MONOXIDE	OXYGEN	ILLUMINANTS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Carrington loam*	66.0	None	20.1	8.6	None	3.7	None
Cecil clay loam*	54.5	None	35.8	6.8	None	2.7	None
Chester loam	67.5	7.8	14.7	4.7	None	3.2	2.1
Hagerstown loam*	58.2	None	30.1	7.5	...	4.2	None
Huntington loam†	64.0	15.9	....	1.8	2.8	...	0.8
Huntington loam	64.8	16.3	7.3	3.9	3.2	3.2	1.2
Huntington loam	66.8	24.2	1.9	3.1	3.0	...	...
Orangeburg fine sandy loam	65.7	12.5	11.8	3.7	None	3.8	2.5
Wabash silt loam†	47.0	25.0	....	3.7	...	...	...
Wabash silt loam*	16.2	None	62.8	15.2	None	5.8	None

* These mixtures supported a heavy growth of blue-green algae.

† There was some air contamination in these samples and consequently a complete analysis was not made.

heavy growth of blue-green algae. The methane of the gases from three of these four soils is lower than the gases from the other soils and the nitrogen is very much higher. The blue-green alga has been identified by N. R. Smith of this bureau as a member of the Chlamydomonos group. When blue-green algae appeared soon after the submergence of the soil the evolution of gas was invariably delayed and the evolution proceeded at a much slower rate than where no algae were present.

The occurrence of carbon monoxide appears to be unusual in marsh gas, and the analysis was checked in several ways. The percentage of carbon monoxide was determined by absorption by cuprous chloride. Under some conditions the alkaline pyrogallol used for the absorption of oxygen, previous to the carbon monoxide determination, may give off some carbon monoxide. However,

several analyses made on gases from other soils in the same manner showed only a trifling absorption, within the limits of experimental errors. The presence of carbon monoxide in the two different samples of gas from the Huntington loam was confirmed by tests with Hoolamite tubes (12). Tests with these tubes on a sample of gas from the Huntington loam showed the presence of a comparatively large quantity of carbon monoxide. Gases from the Wabash and Hagerstown soils, which showed a carbon monoxide absorption so trifling as to be well within the limits of experimental error, gave no test for carbon monoxide by the Hoolamite tubes. The presence of carbon monoxide in gases from submerged soils seems never to have been reported. Löhnis (21), Mulder (23), and Corenwinder (5) report the formation of small quantities of carbon monoxide from manure. Langdon (18) found that the gases of the floaters of the giant help contained from 1 to 12 per cent carbon monoxide.

The composition of the gases has an interesting relation to the composition of the solutions. In all cases carbon dioxide was present in the gas phase in considerable quantities and ferrous iron, manganese, calcium, magnesium, and perhaps other elements were held in solution as bicarbonates. The production of carbon dioxide simultaneously with the reduction of iron, and, probably of manganese, by the action of the anaerobic bacteria on the organic matter accounts for the observed leaching of iron from poorly drained places subjected to the "watery maceration" mentioned by Hilgard (12).

The fact that hydrogen is formed by the microorganisms shows that very strong reducing conditions prevail and free ferric oxide can not be expected to escape reduction under submerged soil conditions.

The quantity of hydrogen sulfide, mercaptans, and other sulfur-containing gases formed is, of course, limited by the quantity of sulfur in the soil. Although there is a very small quantity of sulfur in the evolved gases, or in the solution, there is considerable ferrous sulfide deposited on the sides of the containing bottle and throughout the soil mass. In the Huntington loam, more than three-quarters of the total sulfur had been converted to sulfides after 60 days submergence. This was shown by the quantity of hydrogen sulfide evolved on boiling the submerged soil with dilute hydrochloric acid. Murray and Irvine (24) found a similar precipitation of iron sulfide in the blue muck of the littoral sea bottom when the mud was mixed with decaying organic matter and allowed to stand under water. Rost (27) found sulfides of iron in the lower layers of certain Minnesota peats.

The quantity of organic matter consumed in the production of methane, carbon dioxide, and hydrogen presents an interesting phase of this work. From 100 gm. of Carrington loam 1,365 cc. of gas was produced in about three months. This quantity of gas contained 0.69 gm. methane and 0.2 gm. carbon dioxide. On the assumption that this material is derived solely from the organic matter of the soil it represents about 25 per cent of the organic matter present. A sample of the Huntington showed a more rapid decomposition. A kilo of this soil yielded 250 cc. gas daily. At this rate the organic matter

would be exhausted in about 60 and 70 days. Probably a larger quantity of organic matter went into the solution and into the bodies of the bacteria. Hence the disappearance of soil organic matter under submerged soil conditions may be very rapid, as Hilgard (12) suggests.

The destruction of soil organic matter under submerged soil conditions, as described in the foregoing, appears paradoxical in view of the accumulation of peat in large quantities in horizons submerged in water.

An explanation may be found in the creation of a toxic condition in the peat, as Jeffrey (14) has held. Waksman,[2] however, considers that bacteria do not function in peat because they are unable to subsist on the lignin which is the only carbohydrate left in peat, the cellulose and other carbohydrates having disappeared in the earlier stages of peat formation. The organic matter of the recently submerged soils would contain organic matter other than lignin and therefore support the growth of bacteria. I. C. Feustal of this bureau reports some experiments that are significant in this connection. He submerged three samples of a peat profile and measured the gases given off. After six months no gas had been given off by the samples from the deeper profiles, and the surface sample had given but a few cubic centimeters. The deeper samples were then inoculated with a very active submerged soil and in four months no gas has been given by those samples. Thiessen, Reinhardt, and Johnson (34) consider it proved that bacteria exist and function in peat at all depths. In view of Mr. Feustal's experiments, however, we are forced to conclude that some samples of peat do not contain gas-producing bacteria and furthermore are toxic to such bacteria. Jeffrey (14) holds that yellow spores of bacteria are present even in coals and appear as indestructible as the gold they resemble. He considers that peat in the deeper profile is antiseptic and bacteria are prevented from growth thereby. The toxicity, however, is not sufficient to kill the spores, which may develop under proper conditions. Jeffrey's viewpoint seems to clarify the situation. If spores are present and can germinate, cultures would be obtained when peat samples were put in the proper environment, but it would not follow that bacteria were functioning in the peat.

Gillespie (8) has shown that a strong reduction potential is set up in a few hours in soils submerged by a thin film of water in an open beaker. A disagreeable odor is developed as the reduction potential is set up. This odor is due to hydrogen sulfide or other hydro-sulfides. In this laboratory it has been found that the foul odor develops generally with soils in a short time after an air-dried sample is submerged. These facts suggest two deductions: First, it is apparent that the anerobic bacteria develop rapidly in all soils when the proper conditions obtain; and, second, the sulfur in the soil is rapidly changed to sulfides. The strong reduction potential has a bearing on the toxicity of submerged soil solutions and will be discussed later.

[2] Privately communicated.

TOXICITY OF SUBMERGED SOILS

Livingston (20) showed that bog water usually contains some toxic substance. Dachnowski (6), following the procedure of Breazeale (1) on aerated soil solutions, showed that the toxicity of bog waters is decreased by being shaken with carbon black or calcium carbonate. He also showed that the toxicity of these waters is not due to acidity nor to lack of oxygen.

Experiments reported earlier in this work show that bog waters and submerged soil extracts are high in manganese and ferrous iron and that sulfides are present.

Manganese in the concentrations found is clearly toxic to some plants but not to others. Skinner and Reid (30) found that there was a beneficial effect of manganese on wheat seedlings in concentrations as high as 50 p.p.m. Brenchley (2) found 4 p.p.m. toxic to peas. Gössl (9) states that marsh and water plants contain more manganese than do land plants. Evidently marsh and water plants tolerate much more manganese than do land plants, and other examples could be cited to show that manganese is much more toxic to some land plants than to others.

With ferrous iron the case is very different. Ferrous iron appears to be toxic to land plants in general in concentrations even as low as 3 to 5 p.p.m. There is great difficulty in keeping the iron in a ferrous condition in culture solutions and it is probable that lower concentrations of ferrous iron are toxic. Ruprecht, (28) found that 4 p.p.m. of ferrous iron was toxic to clover seedlings, and Hartwell and Pember (11) showed that 3 p.p.m. was toxic to barley and rye. Skeen (29) found 1 to 2 p.p.m. to be toxic to lupines and Phaseolus. Practically all surface soils containing more than a very small quantity of organic matter develop sufficient ferrous iron to be toxic after being submerged a few days.

Sulfides are produced in all submerged soils. This can be shown by passing the gas over lead acetate paper, by the blackening of the soil mass, and by treatment of the blackened soil with hydrochloric acid with the evolution of hydrogen sulfide. The presence of sulfide-forming bacteria in soils is general, according to Waksman, (35).

Hydrogen sulfide in all concentrations is very poisonous to plants. The concentration in soil solution is never large, being regulated by an excess of iron and other bases in solution. On the other hand, the sulfide concentration is increased by the fact that the solubility of ferrous sulfide is increased by the acidity due to the carbon dioxide in solution. The solution of the Huntington loam after 60 days submergence contained about 14 p.p.m. of soluble sulfides.

Stoklasa (31) reports as high as 273 p.p.m. of alumina in marsh water. Our analyses show only 1 or 2 p.p.m., and the pH of the solutions is such that there can be little alumina in solution. The toxicity of submerged soil solutions therefore cannot be due in general to dissolved alumina.

Nitrites, which are also toxic, develop when well-aerated soils are submerged. Kelley (17) found that when nitrates were added to rice pot cultures and the cultures submerged, nitrites rapidly developed in toxic quantities.

The gaseous constituents present in small quantities such as illuminants, mercaptans, and occasionally carbon monoxide, although toxic, are present in such small quantities as probably to be of little importance.

The toxicity of submerged soils is therefore due to several constituents of which sulfides and ferrous iron are probably the most important. Toxic concentrations of these develop rapidly after the soil is submerged.

DISCUSSION OF RESULTS

The high manganese and iron content of bog waters and of submerged soil solutions has not been emphasized by earlier workers in discussions of the toxicity of such waters. The concentration of manganese observed in submerged soil solutions is not always sufficient to be toxic. With iron and probably hydrogen sulfide, toxic concentrations develop rapidly. It is quite possible that the toxic concentrations of iron, manganese, and hydrogen sulfide observed may be but an effect of a general reducing condition which, in itself, is the main and most quickly effective toxic agent.

Dachnowski (6) has stated that the toxicity of bog waters is not due to the lack of dissolved oxygen. When the surface of the water is open to the air, as in natural conditions, oxygen diffuses rather rapidly from the top downward, as Subramanyan (32) has shown. In such submerged soils, whether the reactions are oxidizing or reducing, will depend upon the rate at which oxygen is consumed by the microbiological activities compared to the rate of oxygen diffusion from the surface. In the experimental work reported here, however, there can be but very little dissolved oxygen after that originally present in the water has been consumed by microörganisms or displaced by the evolution of other gases. The reducing action in submerged soils protected from the air is so intense that hydrogen is commonly given off. The chemical reaction going on under these reducing conditions must be radically different from reactions taking place in aerated soil solutions. The reducing action of submerged soils must in itself be toxic, for it is not to be supposed that the plant can carry on the same normal root absorption in a reducing environment that it does under oxidizing conditions. The lack of dissolved oxygen in submerged soil solutions must be an important factor in studies of the toxicity of such soil solutions. It is beyond the scope of this paper to consider the action of reducing conditions, per se, on the plant. The discussion is therefore confined to such specific effects of this reducing condition as the increase in the concentration of certain inorganic constituents and the production of sulfides.

The experiments performed give us very little idea of the time necessary for soils to become toxic after submergence. In general, toxic concentrations for iron are developed within 8 days. Concentrations of manganese sufficient to be toxic are not always developed in this time. The soils used in the experimental work were air-dried. Since the microörganisms are more active in fresh soils in place, it is probable that soils submerged under field conditions would develop toxic concentrations of manganese and iron in considerably less

than 8 days. Soils submerged for 24 hours generally develop a foul odor, and hydrogen sulfide can be detected in the soil gases.

There is reason to believe that the subsoil solution may differ considerably from that of the surface soil particularly in the iron and manganese content. The subsoil is frequently submerged when the surface soil is not, and part of the surface soil solution has access to the air. Some data recorded have shown that subsoil solutions, taken at the lowest depths of the B horizon, are considerably higher in iron and manganese than the corresponding surface soil solutions.

It is probable that considerable solid matter is transferred by circulation and diffusion from a part of the soil which is submerged to another part having access to the air. Ordinarily this process would work in the direction of the depletion of the lower horizon and the enrichment of upper horizons having both an oxidizing reaction and contact with the solution of the submerged horizon. Mottled spots in poorly drained soils doubtless owe their origin to some such process as this.

Some time ago Comber (4) advanced the hypothesis that plants must be able to absorb colloidal iron from the soil, since the iron found in the soil solution was totally inadequate to account for the iron found in the plant. Plant roots extend into the subsoil and even below. When the water table is high it would be a very easy matter for the solubility of the iron to be so increased as to make considerably more iron available to the plant. The same submerged subsoil conditions which cause more soluble iron should also cause more soluble calcium, magnesium, and manganese.

Poor plant growth on soils that have been submerged but later adequately drained, may be due to lack of essential elements. Hilgard (12) has discussed the destructiveness of this "watery maceration," and the results given here have shown how much more soluble iron, manganese, calcium, and magnesium are produced under submerged soil conditions than under conditions of good drainage. The destruction of the organic matter or certain parts of it, at least, goes on with surprising rapidity.

In poorly drained spots where there is no loss of inorganic elements by slow leaching or diffusion, the loss of organic matter still goes on, and it is apparent that practically all the available organic matter would be consumed by the microörganisms at a rapid rate. Unless organic matter was being added to such a soil by plant growth, the lack of organic matter would be a serious problem if the soil were reclaimed by drainage.

The increase in the solubility of iron under submerged soil conditions has an interesting application in some problems of plant physiology. Gile and Carrero (7) found that rice plants suffering from lime-induced chlorosis when grown in a limestone soil, turned a normal green color when the soil was submerged for 10 days. The correct explanation of this observation was given by Johnson (16), who reasoned that the reducing conditions brought about by submerging the soil so increased the solubility of the iron as to overcome the chlorosis. Pineapples grown on Hawaiian soils high in manganese suffer from chloro-

sis due to lack of available iron. Liberal applications of stable manure to the high manganese soils of Hawaii prevent chlorosis of the pineapples, though this treatment is not so effective on lime-induced chlorosis in Porto Rico. Presumably the beneficial action of stable manure on the Hawaiian soil is due to the reduction of the insoluble ferric compounds to soluble ferrous compounds. Green manuring should also effectively bring about reducing conditions and is a possible clue to the cure of chlorosis under some conditions.

The facts brought out by this work have a possible bearing on the selection of materials for construction of water gardens. Surface soils high in organic matter and also high in manganese and iron will yield a solution high in iron and manganese. Whereas water plants are probably tolerant to higher concentrations of these elements than are land plants, there may be plantings which would be injured by too high concentrations. On the other hand, old leached water gardens may be rejuvenated by the addition of surface soils rich in organic matter, iron, manganese, and other elements that have been leached away. The addition of soils high in manganese and iron but having no organic matter will have little effect in increasing the soluble iron and manganese, since in the absence of organic matter there is no reducing action to increase the solubility of these elements.

The presence of carbon monoxoide in the gases from some submerged soils has a bearing on the unhealthy conditions observed in some swamps. It is not claimed that marsh gas normally contains carbon monoxide but it is believed that under some conditions marsh gas may contain small quantities of carbon monoxide.

SUMMARY

1. Submerged soil solutions are radically different from aerated soil solutions in that they contain high concentrations of iron and manganese. The iron and manganese are present as proto bicarbonates. Submerged soil solutions are also high in calcium and magnesium and contain hydrogen sulfide and other sulfides.

2. The high concentration of iron, manganese, calcium, and mangnesium is caused indirectly by the microbiological action on the organic matter, which produces carbon dioxide along with other gases. It is the carbon dioxide that is mainly responsible for holding the iron, manganese, calcium, and magnesium in solution.

3. In the absence of organic matter the solubility of iron, manganese, calcium, and magnesium is not increased under submerged soil conditions.

4. Soils are not made more acid by sumbergence for short periods except by such acidity as is due to carbon dioxide and bicarbonates.

5. All normal soils containing organic matter, when submerged, eventually produce gas. This gas production is retarded by blue-green algae of the Chlamydomonos group. When blue-green algae are absent, the gas consists mainly of methane and hydrogen. In the presence of the algae the hydrogen

and part of the methane are decomposed. Under these conditions the gas is mainly nitrogen and carbon dioxide with variable quantities of methane.

6. Soil organic matter in aerated soils disappears, in some instances very rapidly, when the soils are submerged.

7. Submerged soil solutions develop toxic concentrations of ferrous iron, sulfides, and commonly manganese. Toxic concentrations of these elements are occasionally developed in bog waters and in solutions of poorly drained soils, and they are invariably developed in submerged soils protected from the air. Soils that have been submerged for a long time may be so profoundly leached of calcium, mangesium, manganese, and iron that they will not support plant growth.

8. Toxic concentrations of ferrous iron and sulfides develop in a few days after submergence. Toxic concentrations of manganese develop somewhat more slowly.

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SOME FERMENTATION CHARACTERISTICS OF VARIOUS STRAINS OF RHIZOBIUM MELILOTI AND RHIZOBIUM JAPONICUM

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The physiological characteristics of the legume bacteria have been studied by a number of investigators in recent years for the purpose of developing a laboratory method which would serve to differentiate the various cross-inoculation groups. Among the more promising physiological tests that have been used for this purpose are those involving the fermentations of carbohydrates and other carbon compounds.

Baldwin and Fred (1) reported that when these organisms were grown on agar media of low buffer capacity and containing brom-thymol-blue, their fermentation characters were sufficiently definite to permit of a separation into groups corresponding to the groups established by cross-inoculation tests. They report further, that in many of the cross-inoculation groups, subdivisions could be established by this method of differentiation. Such subdivisions were made in the alfalfa and clover groups.

Studies on the physiology of these bacteria by Walker (6) showed that there was a wide variation among organisms of the same cross-inoculation groups when they were compared on the basis of their ability to change the reaction of sugar media. It was also pointed out that the variations in this action were as great within a single cross-inoculation group as among the organisms of different groups. The results of the fermentation tests were such that it did not appear justifiable, however, to separate the strains of the organisms into subgroups within the cross-inoculation groups.

Schönberg (5) studied the behavior of 18 strains of legume bacteria, representing a number of the more common species, on nutrient carbohydrate agar containing brom-thymol-blue. These organisms were also studied in solution cultures of the same media without agar. The carbohydrates used were glucose, galactose, xylose, and sucrose. It was found that the solution cultures were better suited for differential diagnostic purposes because of the wider variations in reaction changes produced. Schönberg concluded that the differences in reaction changes were not constant enough to serve as a basis for characterizing and separating the legume nodule bacteria.

It should be noted that Schönberg studied only one strain of some species of this group of bacteria, which was the case for *Rhizobium meliloti*, and only a

few strains of other species. Two strains of *Rhizobium japonicum* were studied. Whether a true picture of the behavior of a group of organisms toward carbohydrate media can be obtained by studying only one or two strains of organisms of that group is very doubtful. In fact, the data presented by Schönberg indicate that there are significant differences in fermentative ability among strains of a single species.

Because of the apparently great variability in the fermentative powers of different strains of single species of the legume bacteria, and since this physiological characteristic has been used as a differential character in the separation of species (2), it was purposed to study this question in further detail in order to ascertain, if possible, the extent of the variations, and also to study the constancy of the fermentative power of individual strains of bacteria.

PRELIMINARY STUDIES

As a preliminary measure of the fermentative characters of these bacteria, a number of individual strains of organisms from the various cross-inoculations groups were grown on agar slants containing different indicators. A basic culture medium of the same composition as that employed by Baldwin and Fred (1) was used. It had the following composition:

Dibasic potassium phosphate	0.5 gm.
Sodium chloride	0.2 gm.
Magnesium sulfate	0.2 gm.
Calcium carbonate	3.0 gm.
Agar	15.0 gm.
Sugar	10.0 gm.
Yeast extract	100.0 cc.
Distilled water	900.0 cc.
Indicator, 0.5 per cent alcoholic sol	5.0 cc.

The reaction of the medium was adjusted to pH 7.0. The indicators used were brom-thymol-blue, and brom-cresol-purple. Glucose, galactose, and mannose were used as sources of energy. The results of these tests were quite interesting, especially when the strains of *Rhizobium meliloti* (2) and *Rhizobium japonicum* were compared. The majority of the *meliloti* strains produced an acid reaction, which resulted in a yellowish color when brom-cresol-purple was used as indicator in the agar. On the other hand, the majority of the *japonicum* strains produced an alkaline reaction and changed the indicator to a deep red. In the first comparison of the groups as a whole, the strains of the two species appeared to be distinctly differentiated by this test, but after closer observation it was noted that not all of the *meliloti* strains produced an acid reaction in the medium. The indicator was not changed in some tubes, and was even a deeper red in other tubes than in the uninoculated control tubes. Likewise, not all of the *japonicum* strains produced an alkaline reaction in the medium, and in such cases the indicator remained unchanged in color or only slightly changed.

The same variations were noted when brom-thymol-blue was used in the medium, but were not so pronounced because of the different pH range of the indicator. The results with this indicator, however, did show marked variations in the fermentative abilities of different strains of single species of bacteria.

Because of the relatively narrow range of a single indicator and the impracticability of making exact pH measurements on agar slopes by comparison with standard indicator solutions, this method of testing the fermentation characters of these bacteria was inadequate and only qualitative in nature.

It was clearly evident from this preliminary work that in order to make a detailed study of the constancy of the fermentative power of a single strain of bacteria and to study the extent of the variability among strains of the same species of this physiological character, the work must be put on a quantitative basis. In order to do this the following method was adopted after a number of preliminary trials had been made.

METHODS USED

A culture medium of the same composition as that employed in the preliminary studies, except that no agar or indicator was added, was prepared and sterilized in flasks containing an amount sufficient for each separate test. Sugar was then added at the rate of 1 per cent of this sterile solution. The sugars used were glucose and galactose. After the sugar was completely dissolved the solution was pipetted into 50-cc. Erlenmeyer flasks, 10 cc. being placed in each flask. The flasks were plugged and sterilized in the autoclave at 15 pounds pressure for 15 minutes. The solutions were then inoculated with a loopful of bacteria taken from a young agar slant. The cultures were incubated at about 28°C. for 100 hours, after which time pH measurements were made by the quinhydrone electrometric method.

The change in hydrogen-ion concentration in the medium was taken as a measure of the fermentative ability of the strain of organisms in that medium. To serve as a control in these tests, two or three flasks of the uninoculated culture medium were incubated along with the inoculated cultures. The difference between the pH of the control cultures and the inoculated cultures was considered to represent the change in reaction produced by the organisms.

The small Erlenmeyer flasks were used instead of test tubes, as the organisms were found to produce greater reaction changes when grown in a shallow layer of solution having a comparatively large surface exposure.

For these studies 23 strains of *Rhizobium meliloti* and 12 strains of *Rhizobium japonicum* were used. The fermentation powers of these organisms were tested in the glucose and galactose media under similar conditions a number of times in order to determine the extent of the variations among strains and the constancy of each individual strain.

RESULTS

The 23 strains of *Rhizobium meliloti* were grown in the yeast-water-glucose medium in eight consecutive tests, and in the yeast-water-galactose medium

in five consecutive tests. The results of these tests are given in tables 1 and 2 respectively. The 12 strains of *Rhizobium japonicum* were likewise grown in the yeast-water-glucose medium in five consecutive tests and in the yeast-water-galactose medium in five tests. The results obtained in these tests are shown in tables 3 and 4.

TABLE 1

Changes in reaction produced by 23 strains of Rhizobium meliloti in eight consecutive tests in a yeast-water-glucose medium

Incubated 100 hours. Results expressed as pH

STRAIN	1	2	3	4	5	6	7	8
Control	6.82	6.55	6.87	6.75	6.76	6.79	6.79	6.79
1	5.84	6.54	6.04	6.00	5.90	6.38	5.97	6.30
2	7.55	7.24	7.77	7.65	7.44	7.25	7.28	7.27
3	6.10	6.29	6.34	6.32	6.28	6.20	6.23	6.25
4	5.85	6.09	6.04	6.17	5.95	6.68	6.69	6.78
5	6.00	6.22	6.21	6.29	6.14	6.25	6.21	6.28
6	5.77	6.12	6.02	6.05	5.89	5.97	5.90	6.03
7	6.52	6.70	6.75	6.79	6.43	6.61	6.49	6.51
8	6.13	6.27	6.31	6.19	6.11	6.31	6.33	6.30
9	5.77	6.20	6.09	6.07	5.93	6.17	6.08	6.18
10	6.23	6.30	6.36	6.32	6.30	6.28	6.21	6.32
11	5.74	6.30	5.78	5.57	5.61	6.13	6.25	6.11
12	5.99	6.06	5.99	6.01	5.95	6.05	6.01	6.00
13	5.84	6.17	6.00	6.05	6.03	6.17	6.00	6.00
14	5.64	6.01	5.71	5.66	5.61	5.87	5.68	5.77
15	5.85	6.16	5.38*	5.59*	5.94	6.05	6.08	6.17
16	6.70	6.67	6.89	6.83	6.80	6.87	6.85	6.82
17	6.78	6.86	7.04	6.88	6.92	6.93	6.93	6.93
18	5.94	6.61	6.02	5.98	5.91	6.25	6.28	6.18
19	5.94	6.22	5.92	5.78	5.99	6.20	6.25	6.17
20	6.58	6.89	6.63	6.49	6.60	6.83	6.87	6.83
21	6.10	6.26	6.32	6.27	6.15	6.32	6.72	6.37
22	6.00	6.30	6.34	6.39	6.06	6.78	6.37	6.47
23	7.57	7.18	7.78	7.57	7.63	7.22	7.27	7.28

* These cultures were probably contaminated.

Constancy of the fermentative powers of individual strains

It can be seen quite readily from these tables that the reaction changes in the consecutive tests with the same organisms on the same medium are very similar. This would seem to indicate that the fermentative powers of individual strains of bacteria are approximately the same in the various consecutive tests, and that the fermentative ability of a particular strain of organisms is a comparatively constant character.

Although the exact amount of change brought about by each strain of bacteria was not the same in all tests, it did bear about the same relationship to the

changes produced by the other strains of the same bacteria. The variations in the conditions under which the experiments were conducted undoubtedly are responsible in large measure for the variations in the exact amount of change produced by an individual strain of organisms in consecutive tests. For example, the reaction of the culture media before inoculation could not be brought to exactly the same pH in each test because of the inherent difficulties in the methods that must necessarily be employed. Variations in the steam pressure during sterilization, and in the length of the sterilization period have

TABLE 2

Changes in reaction produced by 23 strains of Rhizobium meliloti in five consecutive tests in a yeast-water-galactose medium

Incubated 100 hours. Results expressed as pH

STRAIN	1	2	3	4	5
Control	6.87	7.00	6.97	6.97	6.97
1	6.57	6.67	6.41	6.38	6.39
2	7.07	6.96	7.07	7.15	7.10
3	6.14	6.37	6.26	6.24	6.28
4	6.23	6.74	6.26	6.34	6.23
5	6.17	6.40	6.28	6.30	6.32
6	5.97	6.12	6.02	5.99	6.14
7	6.81	6.30	6.68	6.68	6.65
8	6.34	6.34	6.41	6.42	6.42
9	6.13	6.42	6.27	6.30	6.27
10	6.26	6.45	6.23	6.24	6.18
11	6.29	5.77	6.02	5.88	5.90
12	6.08	6.10	6.07	6.07	6.07
13	6.13	6.19	6.14	6.17	6.14
14	5.67	5.48	5.65	5.59	5.57
15	6.11	6.22	6.05	6.05	6.02
16	6.76	6.64	6.65	6.76	6.76
17	6.81	6.67	6.77	6.82	6.84
18	6.24	6.44	6.18	6.20	6.18
19	6.10	6.24	6.27	6.27	6.27
20	6.87	6.47	6.53	6.67	6.62
21	6.18	6.39	6.04	6.41	6.28
22	6.21	6.39	6.42	6.44	6.39
23	6.97	7.14	7.12	7.24	7.14

slight influences upon the final pH of medium. Only by standardization of the methods of procedure can these slight variations be reduced to a minimum. In this work, and especially in the later tests, an attempt was made to standardize the procedure to such an extent as to eliminate, as far as possible, all the variations due to this cause. In spite of these precautions, however, there remained some variations in the media at the time of inoculation.

Other factors responsible in large part for the variability of the reactions of an individual strain of organisms from time to time are the amount of inoculum

used and the stage of growth of the organisms at the time of inoculation. These factors each have a decided influence upon the rate of growth of the organisms, as has been pointed out by Henrici (4) and by Chesney (3). They

TABLE 3

Changes in reaction produced by 12 strains of Rhizobium japonicum in five consecutive tests in a yeast-water-glucose medium

Incubated 100 hours. Results expressed as pH

STRAIN	1	2	3	4	5
Control	6.82	6.55	6.87	6.75	6.76
1	7.60	7.39	7.77	7.77	7.71
2	6.85	7.25	7.09	6.79	6.87
3	7.45	7.58	7.77	7.56	7.79
4	7.55	7.50	7.71	7.54	7.64
5	7.58	7.22	7.22	7.49	7.75
6	7.67	7.32	7.75	7.46	7.66
7	7.71	7.41	7.78	7.62	7.66
8	7.62	7.44	7.78	7.44	7.77
9	7.35	7.30	7.39	7.26	7.33
10	7.63	7.32	7.77	7.60	7.74
11	7.65	7.09	7.78	7.62	7.75
12	7.62	7.36	7.78	7.64	7.73

TABLE 4

Changes in reaction produced by 12 strains of Rhizobium japonicum in five consecutive tests in a yeast-water-galactose medium

Incubated 100 hours. Results expressed as pH

STRAIN	1	2	3	4	5
Control	6.87	6.98	6.86	6.86	6.68
1	7.07	7.53	7.53	7.61	7.58
2	6.96	7.26	7.24	7.17	7.26
3	6.91	7.04	7.10	7.02	6.94
4	7.43	7.51	7.61	7.57	7.58
5	7.37	7.60	7.57	7.54	7.54
6	7.17	7.12	7.58	7.56	7.54
7	6.89	7.68	7.47	7.54	7.53
8	7.29	7.26	7.36	7.54	7.53
9	7.55	7.84	7.79	7.70	7.81
10	6.87	7.71	7.59	7.56	7.56
11	7.31	7.41	7.57	7.46	7.51
12	7.11	6.35	7.59	7.51	7.46

found that when a culture is transplanted during a period of maximum growth rate, it continues to grow at a maximum growth rate in the new medium. But if transplanted at a time when the organisms are in the resting period or in the period of no growth, they show a lag in the new medium. Henrici states

further, that the morphologic variations of bacteria are but outward manifestations of equally profound variations in the physiology of the organisms. He states that there are scattered references in the literature to differences in the fermentative power between young and old cultures.

It is obvious, then, that slight variations in the fermentative power of an individual strain of bacteria would be expected in consecutive tests unless such factors as the size of inoculum and the phase of growth at the time of inoculation, were controlled. And even if the size of inoculum were accurately controlled, not all of the cells would grow at the same rate, and there would be a selection of the most rapidly growing strain from the inoculum as has been shown by Henrici (4). It would seem, then, that these factors would influence considerably the extent of the fermentation in a limited incubation period.

It is also very obvious that these factors could not be absolutely controlled for routine work. It would be almost impossible to inoculate fermentation media in a number of consecutive tests with a large number of strains of bacteria, variable as they are in physiology, with the same number of organisms in the same phase of growth in each case. In the studies reported here, however, an attempt to standardize these conditions has been made, by inoculating with approximately the same size of inoculum, as measured by a loop, and by transferring from cultures of approximately the same age. The organisms of course were not necessarily in the same phase of growth.

In spite of the fact that all the conditions of the various tests could not be controlled and were not absolutely indentical it seems very evident from the data that the fermentative characters of an individual strain of these bacteria are fairly constant.

The fact that individual strains of bacteria reacted similarly in consecutive tests, even though the environmental conditions were not absolutely identical, seems to substantiate further the conclusion drawn in the foregoing and give additional weight to it. It seems very probable that if all environmental conditions, such as reaction and composition of the medium, temperature and time of incubation, size of inoculum and stage of growth of the bacteria used, could be controlled in such a way that all tests were conducted under absolutely identical conditions, the results obtained in the fermentation of a sugar by a particular strain of bacteria would be very nearly the same in consecutive tests.

Variations in the fermentative powers of different strains

These data also indicate quite clearly that there are large variations in the fermentation characters of different strains of the same species of bacteria. This is shown by the results of the tests in glucose medium with practically all the cultures, but especially so with cultures 1, 2, 11, 14, 16, 17, and 23 of *Rhizobium meliloti* and with cultures, 1, 2, 9, and 12 of *Rhizobium japonicum*. In every case, cultures 2 and 23 of the *meliloti* species produced a very alkaline reaction in the glucose medium; 16 and 17 changed the reaction but slightly,

making it more alkaline in all but one case; 1, 11, and 14 produced a strongly acid reaction in the medium. In the third and fifth tests on the glucose medium, strain 14 produced a reaction over 100 times as acid as that produced by strain 23. Other strains produced reactions intermediate between these extremes. In the case of the *japonicum* strains, 2 changed the reaction but

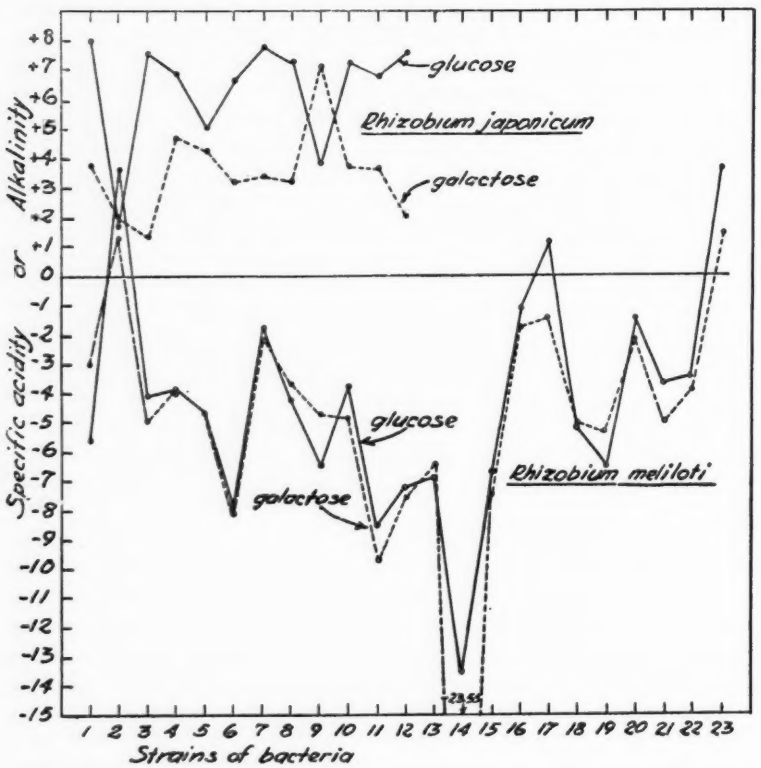

FIG. 1. VARIATIONS IN AMOUNTS OF SPECIFIC ACIDITY OR SPECIFIC ALKALINITY PRODUCED BY 23 STRAINS OF *Rhizobium meliloti* AND BY 12 STRAINS OF *Rhizobium japonicum* WHEN GROWN IN YEAST-WATER-GLUCOSE AND YEAST WATER-GALACTOSE MEDIA

slightly in the glucose medium; 1 and 12 produced a rather strongly alkaline reaction, and 9 produced an intermediate reaction. Some of the strains of this species produced reactions almost ten times as alkaline as those produced by strain 2.

The variations between strains in ability to alter the reaction of media is also shown when the data for the fermentation of galactose are compared.

Strains 2 and 23 of the *meliloti* species made the medium more alkaline in all but one case, whereas strain 14 brought about a very strongly acid reaction of the medium. Strains 16 and 17 produced only very slight changes in the reaction of this medium, making it slightly more acid than the control. Other strains varied in their fermentative powers between these two extremes.

In the case of strain 3 of the *japonicum* species a very slight change in the galactose medium was produced. Strain 9, however, produced a fairly alkaline reaction in this medium. The other strains varied between these extremes in reaction effects.

The extent of the variations in the fermentative powers of different strains of these two species of *Rhizobium* in glucose and galactose media is further illustrated in figure 1. In this figure, graphs have been made of the averages of the actual amounts of acidity or alkalinity produced in the media. Because of the fact that when results are expressed in terms of pH, as they are in the tables already referred to, the figures are reciprocals of the logarithms of the numbers. When data of this type are plotted the differences between strains appear to be less variable than they really are. For this reason, the actual amounts of acidity or alkalinity produced have been plotted in terms of specific acidity or specific alkalinity as suggested by Wherry and Adams (7), in order that a true comparison of the fermentative abilities of the different strains of bacteria may be made.

These graphs show very clearly the high degree of variation between strains of the same species in the ability to ferment glucose and galactose.

Effects of growth upon different sugars

Another interesting relationship brought out by these data and shown in the graphs, is the fact that the various strains of *Rhizobium meliloti* ferment glucose and galactose with about the same comparative results in the changes in reaction. On the other hand, the strains of *Rhizobium japonicum* do not appear to ferment these two sugars in the same manner.

Comparison of the fermentative powers of different species

One very important fact shown by these data is that although the reactions produced by the majority of the *japonicum* strains are much more alkaline than those produced by the majority of the *meliloti* strains, this was not true in all cases. For example, the reactions produced by the *meliloti* strains 2 and 23 were as alkaline as some of the *japonicum* strains in both of the sugar media. *Meliloti* strain 17 produced a reaction in the glucose medium almost as alkaline as some of the *japonicum* strains.

These results would certainly indicate that the fermentation test with glucose or galactose and under conditions similar to those followed in these experiments would not serve to give a distinct separation of organisms of these two species of legume bacteria. It would seem that this test alone is hardly sufficient to

serve as a differential character in studies with these two species. It is entirely possible that after further studies of the physiological characteristics of these organisms such a test, when supplemented by others, will prove of distinct differential value and aid considerably in the study of the legume bacteria.

SUMMARY AND CONCLUSIONS

Twenty-three strains of *Rhizobium meliloti* and twelve strains of *Rhizobium japonicum* were studied to ascertain the constancy of their fermentative characteristics and also the extent of the fermentative variations among individual strains of the same species. These organisms were grown in yeast-water-glucose and yeast-water-galactose media in five or more consecutive tests. The change in hydrogen-ion concentration in the media was taken as a measure of the fermentative ability of the strain of organisms in that medium.

The results indicate that the fermentative powers of individual strains of these bacteria were approximately the same in the various consecutive tests, and that the fermentative ability of a particular strain of organisms is a comparatively constant character.

The data also indicate clearly that there are large variations in the fermentation characters of different strains of the same species of legume bacteria. Some of the *meliloti* strains produced a distinctly alkaline reaction in the media and others produced a strongly acid reaction in media of the same composition. Some strains made the media a hundred times as acid as other strains of the same species. Some of the *japonicum* strains produced a very slightly alkaline reaction in the media whereas others produced a reaction almost ten times as alkaline.

The various strains of *Rhizobium meliloti* fermented glucose and galactose with about the same comparative results. On the other hand, the strains of *Rhizobium japonicum* did not appear to ferment these two sugars in the same manner.

The majority of the *japonicum* strains produced a much more alkaline reaction than the majority of the *meliloti* strains, but this difference was not true for all individual strains.

These results indicate that the fermentation test with glucose and galactose and under conditions similar to those followed in these experiments would not serve to give a distinct separation of organisms of these two species of legume bacteria.

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A COMPARISON OF SOME NODULE FORMING AND NON-NODULE FORMING LEGUMES FOR GREEN MANURING[1]

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The customary assumption that leguminous crops are best for green manuring is largely based on the known ability of such crops to fix nitrogen from the air in conjunction with certain soil bacteria. The fact that such a fixation is taking place is manifested by the presence of characteristic nodular growths on the roots. The wide natural distribution of the organisms which produce these nodules and help fix nitrogen is responsible for the quite general presence of these growths. The absence of nodules from the roots of legumes is not always an indication that the plant and bacterium are incapable of forming a beneficial alliance, because the proper strain of the bacterium may be absent from the soil; but there are, however, plants classified as legumes which apparently never produce these nodules. A list of some of these plants given by Leonard (5) includes species of *Cassia* some of which are indigenous to the United States and on which it has been possible to make many observations. Since there are nodule-bearing species included in the genus *Cassia* it is quite desirable to mention that this work deals with *Cassia occidentalis* and *Cassia tora*. For these two legumes we have not found any authentic reports which show that they have ever been found with nodules on their roots in this country. Keuchenius (4) reports the presence of nodules on the roots of *Cassia occidendentalis*, yet correspondence in 1923, 1926, and 1927 with the Institut voor Plantenziekten through Dr. Carl Hartley and the Algemeen Proefstation voor den Landbouw, both of Buitenzorg, Java, through the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, U. S. Department of Agriculture, has revealed no evidence to support the statement that nodules occur on either this species or on *Cassia tora*.

The absence of the ability to form nodules does not preclude the possibility of the plant fixing nitrogen, since there is some evidence in the work of Friesner (3) and of Feher and Bokor (2) that organisms inhabit the swollen roots of the honey locust, *Gleditsia triacanthos*, and these may have a function similar to that of the nodule bacteria. Roots of the *Cassia* species under discussion are

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usually black or greenish yellow and rather thick. Information somewhat opposing this theory is given by Nobbe, Schmid, Hiltner, and Hotter (7) and by McDougall (6), all of whom ascribe lack of nodules on the roots of certain members of the sub-family *Caesalpinaceae* to the presence of thick-walled root hairs which prevent the entrance of the organisms.

Whether nodules are produced or not, whether nitrogen fixation takes place or not, both of these species of *Cassia* have been employed in green manuring experiments. The most favorable report comes from Allan (1), in which he states that a uniformly greater wheat crop over a period of 10 years was obtained after crops of *C. occidentalis* than after crops of *Crotalaria juncea*. Van Helten (9) and Rant (8) employed these two species of *Cassia* in his experimental work with green manuring crops.

OUTLINE OF EXPERIMENTAL WORK

For the purpose of determining the green manuring value of *C. occidentalis* and *C. tora*, seeds of these species and of other legumes were planted May 27, 1927, at the Coastal Plain Experiment Station, McNeill, Misssissippi. The soil on which these were sown is classified as Orangeburg sandy loam. It is somewhat acid, low in nitrogen, fairly uniform in texture, and practically level. Each kind of seed was planted in rows 44 inches apart, 66 feet long, on $\frac{1}{10}$-acre plots. An aisle 44 inches wide was provided as a dividing line between the plots. In addition to the seeded plots three unseeded ones were laid out for comparison. The crops, plot numbers, rotation, and rates of seeding are given in table 1.

The plots were located as shown in the following diagram:

North

5	4	3	2	1
8	7	6		

East

Leguminus crops

The crops of the 1927 summer period grew well, particularly *C. tora*, which on August 19 practically hid the soil in the 44-inch rows; *Sesbenia macrocarpa*, which was 10 feet high at this time; and *Crotalaria spectabilis* which gave evidence of a heavy crop. No nodules were found on the roots of the two species of *Cassia* but all other legumes in this experiment showed nodulation; *S. macrocarpa* roots were especially well laden with nodules. On September 24 these crops were harvested from half of each plot, one-twentieth of an acre, for it was planned to turn under not only stubble but also total crop. The data obtained from these crops are given in table 2.

It is apparent that the outstanding crop yields were produced by *C. tora*

and *Crotalaria spectabilis*. Table 2 indicates that these legumes were also superior to the others in percentage of nitrogen. Otootan soybeans did not compare favorably with the other crops either with respect to nitrogen or amount of crop produced.

All nitrogen analyses were made on dry tops of the plants. Roots were not included, since their effect should be evidenced by subsequent indicator crops.

TABLE 1

Arrangement of crops

PLOT NUMBER	1927 SUMMER CROP	ACRE RATES OF SEEDING 1927 SUMMER CROPS	1927–28 1928–29 WINTER CROPS	1928 SUMMER CROPS
		pounds		
1	*C. occidentalis*	100	Oats	Corn
2	None	...	Oats	Corn
3	*C. tora*	60	Oats	Corn
4	None	...	Oats	Corn
5	*Sesbania macrocarpa*	65	Oats	Corn
6	Otootan soybean	80	Oats	Corn
7	None	...	Oats	Corn
8	*Crotalaria spectabilis*	85	Oats	Corn

TABLE 2

Crop yields and analyses of legume crops

PLOT NUMBER	CROP	MOISTURE IN GREEN HAY	ACRE YIELD OF DRY HAY	N* IN DRY HAY	N IN AN ACRE OF HAY
		per cent	*pounds*	*per cent*	*pounds*
1b†	*Cassia occidentalis*	52.5	3,110	1.50	46.6
3b	*Cassia tora*	62.5	4,587	1.68	77.1
5b	*S. macrocarpa*	62.5	3,945	1.20	47.3
6b	Soybean	70.0	2,131	1.60	34.1
8b	*Crotalaria spectabilis*	77.5	4,296	1.65	70.9

* Analyses made by Daniel Ready.

† Designation given to part of plot from which crops were removed.

Nonlegume indicator crops

Oats were planted on all plots, October 18, 1927, at the rate of 2 bushels an acre. On March 12, 1928, it was noticed that these oats had made the best growth on the *S. macrocarpa* plot; they were light green on the *Cassia* plots and dark green on all other plots. Hogs grazing on the oats apparently preferred material from the plots that had been fallowed. A complete report of the condition of the crop at this time is given in table 3.

The 1927–28 crops of oats from the fallow plots were superior in most respects to the crops from the other plots. This may be due to treatment, although the amount of material added to them was small and consisted only of young plants

TABLE 3

Condition of oats crop, March 12, 1927

PLOT	LEGUME PLOWED UNDER	HEIGHT OF OATS	STOOLING	COLOR
		inches		
1a	*Cassia occidentalis*	6	Little	Light green
1b	*Cassia occidentalis* stubble	5	Little	Light green
2	None	6	Medium	Dark green
3a	*Cassia tora*	7	Medium	Light green
3b	*Cassia tora* stubble	6	Little	Light green
4	None	8	Much	Dark green
5a	*Sesbania macrocarpa*	11	Much	Dark green
5b	*Sesbania macrocarpa* stubble	7	Medium	Dark green
6a	Otootan soybean	7	Medium	Dark green
6b	Otootan soybean stubble	6	Little	Dark green
7	None	7	Medium	Dark green
8a	*Crotalaria spectabilis*	9	Much	Dark green
8b	*Crotalaria spectabilis* stubble	6	Medium	Dark green

TABLE 4

Data on indicator crops following legumes

	OATS 1927–28			OATS 1928–29	CORN 1928, YIELD PER ACRE	
	Average height 4/30/28	Hay yield per acre	N in oat hay	Hay yield per acre	Stover	Grain
	inches	*pounds*	*per cent*	*pounds*	*pounds*	*pounds*
Cassia occidentalis crop	34	2,560	0.80	800	2,320	880
Cassia occidentalis stubble	33	2,475		720	2,325	450
None	42	4,987	1.16	1,150	1,788	773
Cassia tora crop	38	3,000	1.05	1,200	2,350	750
Cassia tora stubble	34	2,138		920	2,182	515
None	48	4,220	1.27	1,050	1,900	520
Sesbania macrocarpa crop	48	3,500	0.94	720	1,842	474
Sesbania macrocarpa stubble	43	3,600		760	2,320	640
Otootan soybean crop	40	2,860	1.07	1,100	2,111	888
Otootan soybean stubble	36	2,093		900	1,455	636
None	46	3,600	1.15	1,200	1,700	802
Crotalaria spectabilis crop	45	3,460	0.87	1,040	1,879	758
Crotalaria spectabilis stubble	38	3,218		740	2,030	636
Averages:						
Crops (nodule bearing)	44	3,273	0.96	953	1,944	707
Stubble (nodule bearing)	39	2,970		800	1,935	637
Crops (Cassias)	36	2,780	0.93	1,000	2,335	815
Stubble (Cassias)	34	2,306		820	2,254	483
Crops (all legumes)	41	3,076	0.95	972	2,100	750
Stubble (all legumes)	37	2,705		808	2,062	575
None	45	4,269	1.19	1,133	1,796	698

of *Richardsonia scabra* and cockle burs which grew during the periods the soil was not cultivated. The yield of oat hay was not only higher in the fallow plots but this hay contained more nitrogen.

The same general trend is noted in the oat crop harvested in 1929, but the yields and the differences between yields are much smaller. In only one case in either of the oat harvests is the crop following the stubble greater than that following the corresponding crop turned under, and this seems significant because it is constant in both the 1928 and 1929 figures for *Sesbania macrocarpa* plots. The same condition obtains in the corn data. It is quite possible that the proximity of pine trees to the part of the plot into which the whole crop was plowed or the abundance of cellulosic material which occurs in the plants of *Sesbania* was responsible for this condition.

Data on corn stover do not present very significant correlated differences but the corn grain yields follow the same general trend of the preceding and succeeding oat crops.

SUMMARY

In a comparison of legumes not bearing nodules with legumes bearing nodules in a green manuring experiment at McNeill, Mississippi, on slightly acid Orangeburg sandy loam, *Cassia tora*, one of the former, gave the greatest yield of dry hay, whereas Otootan soybeans, one of the latter, gave the least yield of dry hay.

The first indicator crop of oats was light green on the *Cassia* plots whereas it was dark green on the other plots. The oats from the fallow plots, for some reason not definitely determined, were superior in nitrogen, weight, and height. A subsequent crop of oats grown on the plots during the next winter season followed the same general trend in weight.

The second indicator crop, which was corn, gave data on stover that were not very consistent and on corn grain that practically followed the same crop weight trend as the oats.

Turning under the whole crop of legumes gave better results with indicator crops than did just stubble. Considering the data as a whole, no decided differences are shown between the after-effects of noduled or non-noduled legumes.

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NON-RECIPROCAL CROSS-INOCULATION OF LEGUME NODULE BACTERIA[1]

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Until recently 11 groups of legume nodule bacteria were recognized, based upon their tendency to produce nodules upon certain species of legume plants. An interchangeability was supposed to exist between the members of the same group so far as the nodule forming function is concerned. Furthermore, this phenomenon, known as cross-inoculation, was thought to occur only between members of the same group. An additional group was suggested when Whiting, Fred, and Helz (5) reported studies with Wood's clover (*Dalea alopecuroides*) nodules bacteria in which they found that this organism did not produce nodules upon alfalfa and sweet clover plants and that the Wood's clover plant was not infected by bacteria belonging to other cross-inoculating groups. No report is given of any trials with the Wood's clover organism in which attempts were made to inoculate legumes other than alfalfa and sweet clover.

Leonard (2) and Sears and Carroll (3) have shown that the cowpea group of organisms cannot be considered entirely separate from the soybean bacteria since there is some interchangeability between the two groups. The latter workers have concluded, however, that this interchangeability is not complete, since some cowpea nodule bacteria have been found which failed to produce nodules on the soybean plant. In other words, cross-inoculation between cowpea and soybean nodule bacteria is not completely reciprocal.

Stevens (4) and Wright (6) have found that it is possible to divide the groups of nodule bacteria into strains of biotypes on the basis of serological and cultural and physiological characteristics. The studies of Sears and Carroll (3) indicate that strains of cowpea bacteria apparently differ also in their capacity to produce nodules on the soybean plant.

WOOD'S CLOVER NODULE BACTERIA INFECT THE GARDEN BEAN

When Illinois farmers became interested in the use of Wood's clover for soil improvement, the problem of inoculation naturally presented itself. Conse-

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quently, inoculation studies with this legume were undertaken and as a result it was found that the Wood's clover plant did not produce nodules when inoculated with the nodule bacteria from any of the common legumes.

In order to establish the fact that this legume does not belong to any of the known cross-inoculation groups, it seemed necessary to conduct additional tests in which legumes representing each group were inoculated with the Wood's clover nodule organism. As a result of such studies, it was found that garden and navy bean plants produced nodules when inoculated with the Wood's clover nodule bacteria. In addition, it was found that a culture isolated from the garden or navy bean nodule, and which had resulted from the use of a Wood's clover culture, infected the Wood's clover plant. No legumes studied belonging to other cross-inoculating groups were infected by the Wood's clover nodule organism.

TABLE 1

Cross-inoculation with Wood's clover nodule bacteria

CULTURE	LEGUME	NODULATION
Sweet clover	Wood's clover	−
Red clover	Wood's clover	−
Soybean	Wood's clover	−
Cowpea	Wood's clover	−
Garden bean	Wood's clover	−
Navy bean	Wood's clover	−
Wood's clover	Wood's clover	+
	Sweet clover	−
	Red clover	−
	Soybean	−
	Cowpea	−
	Garden bean	+
	Navy bean	+

EXPERIMENTAL METHODS

The conditions under which cross-inoculation studies are conducted determine the reliability of the results obtained. Consequently, great care was used to prevent the probability of error due to contamination from any source. The plants were grown in a greenhouse devoted exclusively to inoculation studies, and into which no soil was allowed to be taken. As a result, the controls were uniformly free from nodules. The cultures used were obtained from our own isolations and from transfers obtained from other investigators.

In order to be sure that mixed cultures were not being used, single cell isolations were made in addition to the usual tests employed in pure culture studies.

In addition to the technique described by Sears and Carroll, the method of Garman and Didlake (1) was used.

The data presented in table 1 confirm the results of Whiting, Fred, and

Helz in that it was impossible to produce nodules upon the Wood's clover plant by inoculation with the nodule bacteria of any of the common legumes. However, it was found that cultures of the Wood's clover bacteria were able to infect plants in the garden bean group.

Previously it has been assumed that if the nodule bacteria of one kind of plant infect another, their nodule organisms are mutually interchangeable. Sears and Carroll (3) reported, however, that such a condition does not always obtain in the case of certain cowpea and soybean organisms, and in later studies they found that the cowpea plant is capable of working in symbiosis with bacteria from the nodules of a great number of legumes whose nodule organisms do not in all cases cross-inoculate among themselves. Here is a

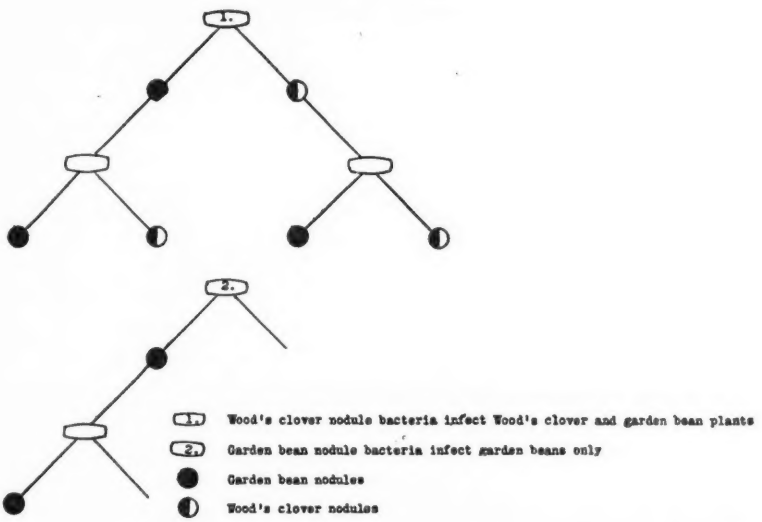
1.
2.
1. Wood's clover nodule bacteria infect Wood's clover and garden bean plants
2. Garden bean nodule bacteria infect garden beans only
Garden bean nodules
Wood's clover nodules

FIG. 1. RELATION BETWEEN GARDEN BEAN AND WOOD'S CLOVER NODULE BACTERIA

case in which nine garden bean cultures failed to produce nodules upon the Wood's clover plant, whereas seven cultures of Wood's clover bacteria infected the navy and garden bean plants. It would appear, therefore, that the interchangeability of nodule bacteria, as far as the nodule forming function is concerned, is not necessarily reciprocal in the case of Wood's clover and the members of the garden bean group.

It was of interest to find, however, a peculiar root development in some instances where ordinary garden or navy bean inoculation had been applied to Wood's clover plants. A knotted condition appeared upon the roots, which suggested the possibility of infection, but a number of attempts at isolation of nodule bacteria from these roots resulted in failure. This unusual root development was not observed under any other conditions.

Naturally, the question then arose as to whether bacteria isolated from the nodules on the garden or navy bean, and which had resulted from infection by Wood's clover bacteria, would still have the ability to infect Wood's clover plants. Sixteen such cultures were isolated and subsequently tested upon both garden beans and Wood's clover and in all cases with positive results. It appears, therefore, that plant passage did not alter the organism so far as its ability to form nodules on Wood's clover is concerned. Furthermore, the agar plate colonies and agar slant growth were typical of the Wood's clover organism.

Figure 1 shows diagrammatically the methods and results which are typical of those obtained from the use of seven Wood's clover and nine garden bean cultures. When obtained from picked colonies after repeated platings, as well as from direct isolation from nodules, the cultures were uniformly consistent in their behavior with regard to infecting Wood's clover and garden bean plants.

TABLE 2

Nitrogen fixation by garden bean plants

CULTURES		NITROGEN†			
Number	Kind	Tops	Roots	Total	Fixed
		mgm.	*mgm.*	*mgm.*	*mgm.*
1	Wood's clover	176.1	94.7	270.8	90.8
43	Wood's clover	163.3	106.0	269.3	89.3
1	Garden bean	300.1	95.9	396.0	216.0
30	Garden bean	263.3	111.5	374.8	194.8
57*	Garden bean	148.6	103.4	252.0	72.0
10*	Garden bean	157.4	98.4	255.8	75.8
	Check	110.4	69.6	180.0	.....

* Cultures 10 and 57 were isolated from garden bean nodules which resulted from infection caused by Wood's clover nodule bacteria.

† Average of three replicates.

Since the bacteria that were obtained from widely different sources gave similar results, it seemed unlikely that this phenomenon was the result of impure cultures. The additional fact that cultures resulting from single cell isolations exhibited the same characteristics lends further evidence to prove a non-reciprocal interchangeability of garden bean and Wood's clover nodule bacteria.

Since recent investigations have shown that strains of legume nodule organisms differ in their capacity fo fix atmospheric nitrogen and since two unlike organisms had produced nodules upon the garden bean, it seemed desirable to determine the comparative affectiveness of Wood's clover and garden bean nodule bacteria for nitrogen fixation. The results of such a test are reported in table 2.

Considering the nature of the experiment, the data are surprisingly consistent. The amounts of nitrogen fixed by the two cultures of Wood's clover

nodule bacteria are practically the same and differ but little from those found in the plants infected by cultures 10 and 57, which were isolated from garden bean nodules resulting from the use of Wood's clover bacteria.

Although it would not have been surprising had the two garden bean cultures differed in their nitrogen fixing ability, the results obtained from their use were very similar. They differed greatly, however, from the other cultures, which fixed an average of 82 mgm. per jar when functioning symbiotically with the garden bean plants. The garden bean plants infected by the original garden bean cultures contained 205.4 mgm. more nitrogen than the controls.

From these data, it is evident that even though abundant nodule formation resulted from the use of Wood's clover nodule bacteria on garden bean plants, the amount of nitrogen fixed was less than one-half as much as that fixed under conditions in which the garden bean plants were infected by garden bean bacteria. It is apparent, therefore, that in this case, at least, the degree of nodulation cannot be taken as an index of nitrogen fixation.

The appearance of the plants before harvest clearly showed that those inoculated with the garden bean nodule organism were getting more nitrogen than any of the other plants. They were dark green and had a healthy and thrifty appearance, whereas the crop in jars to which Wood's clover organisms had been added looked but little better than the check. In fact, even up to harvest time, there was some doubt as to whether nodulation had occurred. Examination at time of harvest showed an abundant nodule formation even though the plants were yellow and unthrifty.

SUMMARY

1. The nodule organisms from none of the common legumes studied ordinarily produced nodules on the Wood's clover plant.

2. Pure cultures of Wood's clover nodule bacteria produced nodules upon garden and navy bean plants.

3. Pure cultures of organisms isolated from the garden or navy bean infected Wood's clover, provided the bean nodules resulted from infection by Wood's clover nodule bacteria.

4. When judged by nitrogen fixation, the bean cultures were more effective upon the bean plant than the Wood's clover cultures, even though each culture produced abundant nodule development.

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